Current Drug Targets for Antihyperlipidemic Therapy

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Abstract: Elevated lipid level is supposed to be one of the main risk factors of atherosclerosis and related cardiovascular diseases and stroke (and is connected to mortality and morbidity). Therefore, lipid lowering is one of the major approaches in prevention of coronary heart diseases and stroke. Though drugs of various categories acting through different mechanisms are available in the antihyperlipidemic therapy, there are still a few problems associated with the currently available lipid lowering drugs. Therefore, medicinal chemists worldwide are designing, synthesizing and evaluating a variety of new molecules for antihyperlipidemic activity to address these problems. One of the important approaches to this is identifying new drug targets for antihyperlipidemic activity. This review summarizes nineteen recently identified and currently being exploited targets for the ongoing research by researchers world over to discover novel leads as potential drugs for antihyperlipidemic therapy.

Keywords: Antihyperlipidemic, antiatherogenic, drug targets, reverse cholesterol transport.

1. INTRODUCTION

Atherosclerosis was relatively uncommon about 100 years ago mainly due to physically active lifestyle. As the 20th century progressed, high-fat diets, sedentary lifestyles, cigarette smoking and urbanization, have combined to increase the prevalence of hypercholesterolemia, as well as, coronary heart disease (CHD) and stroke, throughout the developed world. Researchers have established a link between atherosclerosis and elevated blood cholesterol levels. Therefore, lipid lowering is one of the major approaches in prevention of cardiovascular diseases, which still remain the major cause of mortality in modern societies [1, 2]. The probable biological risk factors for cardiovascular diseases include lipid abnormalities, glucose intolerance, insulin resistance, hypertension, abnormalities in hemocoagulation and serum hyperhomocysteinemia. Hypercholesterolemia is an important cause of coronary heart disease (CHD) [3] and the role of cholesterol in the formation of atherosclerotic lesions has been confirmed, experimentally [4].

2. MAJOR FAMILIES OF BLOOD (PLASMA) LIPOPROTEINS

The various constituents often collectively referred as lipids, include fatty substances, fatty acids, cholesterol, triglycerides as well as lipoproteins (which are the combination of the later two) [5-10]. The lipoprotein classification based on triglyceride and cholesterol contents is represented in Fig. (**1**). Apolipoproteins; the proteins that carry lipids in plasma are also important (Table **1**).

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3. LIPOPROTEIN METABOLISM

It is important to clearly understand the lipid and lipoprotein metabolism in which liver and intestine are the main organs involved [11]. Lipid transport in plasma is facilitated by apolipoproteins, which solubilize lipoproteins in the plasma and transport them during their metabolic process. Apolipoproteins are divided into five families apo-A to apo-E. The apo-A family associates with HDL and chylomicron transport; apo-B family associates with VLDL, LDL, IDL transport; apo-C family associates with IDL, VLDL and HDL transport; apo-D family associates with HDL transport, while the apo-E associates with the transport of chylomicron remnants, VLDL, IDL and HDL. These activated apolipoprotein-lipid complexes then undergo lipolysis with a variety of enzymes namely, lipoprotein lipase [12], hepatic triglyceride lipase [13], lecithin cholesterol acyl transferase (LCAT) [14], cholesterol ester transfer protein (CETP) [15], microsomal triglyceride protein (MTP) [16], and acyl CoA transferase (ACAT) [17] (Table **1**).

4. PATHOPHYSIOLOGY OF HYPERLIPIDEMIA

For the choice of treatment regimens, an understanding of the biology of the lipoproteins and the pathophysiology of hyperlipidemic states is very useful [18], in deciding the drug therapy for the treratment of hyperlipidemia and atherosclerosis.

1. Lipids are taken up as chylomicrons, which after removal of triglycerides by lipoprotein lipase get transferred to liver as chylomicron remnants. The triglycerides are stored in the adipose tissue. This is the *exogenous pathway* for uptake of dietary lipids.
2. Liver secretes VLDL in plasma where triglycerides are removed by lipoprotein lipase. The resultant LDL is cleared from plasma through LDL mediated endocytosis.

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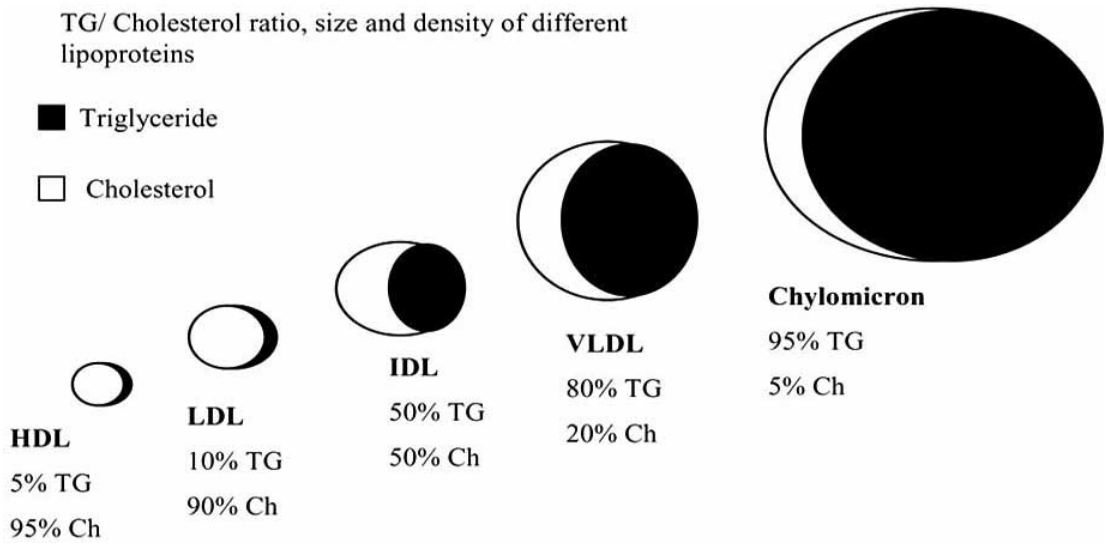


Fig. (1). Lipoprotein classification.

This is the *endogenous pathway* for distribution of cholesterol esters from liver to target cells.

3. Cholesterol from atherosclerotic plaques or dead cells or cell membrane tumors is sent back to liver to be either excreted into the feces *via* bile or reincorporated into the LDL pool. This is called as *reverse cholesterol transport pathway* [19]

4. Liver synthesizes 2/3rd of the total cholesterol made in the body. The rate limiting enzyme is 3-hydroxy-3-methylglutaryl coenzymeA (HMG-CoA) reductase and provides feedback regulation by controlling the cholesterol concentrations in cells.

5. The liver converts cholesterol into bile acid salts, of which small amount is excreted through intestine and rest is reuptaken.

Based on the above knowledge of the pathophysiology and metabolism of lipids and lipoproteins, the currently available drugs for treatment of hyperlipidemia mainly include the HMG-CoA reductase inhibitors; (namely the statins), bile acid sequestarants, fibrates, lipoprotein lipase stimulants, niacin and cholesterol absorption inhibitors.

Though drugs of various categories acting through different mechanisms are available in the antihyperlipidemic therapy, there are a few problems associated with it as enlisted below;

1. New drugs are required covering the hitherto untreatable cases of Type II hyperlipidaemia, wherein drugs like clofibrate, nicotinic acid, d-thyroxin etc., have been used without much success.

2. Also new drugs are needed to be discovered, which will be able to block the stimuli that lead to the formation of an atherosclerotic lesion.

3. Furthermore, specific drugs are needed to be developed, which could bring about regression of the already existing atherosclerotic lesions, in the blood vessels.

4. The most widely used "statins" suffer from limitations like, intolerance and adverse effects, ineffective or only partially effective in lowering of cholesterol levels and achieving only up to maximum 40% risk reduction. Finally, their use in therapy is still expensive.

Therefore, medicinal chemists worldwide are designing, synthesizing and evaluating a variety of new molecules for antihyperlipidemic activity to address the above problems.

One of the important approaches to this is identifying new targets for the evaluation of antihyperlipidemic activity of potentially bioactive molecules. Following are some recently identified and currently being exploited targets for the ongoing research worldover to discover novel leads and eventually new drugs for antihyperlipidemic therapy.

5. CURRENT DRUG TARGETS FOR ANTIHYPERLIPIDEMIC THERAPY

5.1. Inhibition of Cholesterol Absorption

Intestinal cholesterol absorption begins with the micellar solubilisation of both dietary and biliary cholesterol in the lumen of the small intestine. In general, approximately two thirds of intestinal cholesterol is absorbed from the bile and the remaining one third is derived from the diet. The cholesterol is then transferred from the micelles to the surface of the brush border membrane of the enterocytes, and finally into their cytoplasmic compartment. Cholesterol moves to the endoplasmic reticulum, where it may be esterified by the enzyme acyl CoA: cholesterolacyltransferase (ACAT) to form cholesterol esters. Free cholesterol and cholesterol esters are packaged into chylomicrons, which are then secreted into the mesenteric lymph. Once into the circulation, the chylomicrons and their remnants are rapidly cleared by the liver. The consequences of cholesterol absorption inhibition include decreased cholesterol delivery to the liver, reduced hepatocyte cholesterol stores, decreased low density lipoprotein production, increased LDL clearance and subsequently, decreased LDL cholesterol levels [20, 21].

Table 1. Apolipoproteins and Enzyme/s Involved in the Lipid Metabolism

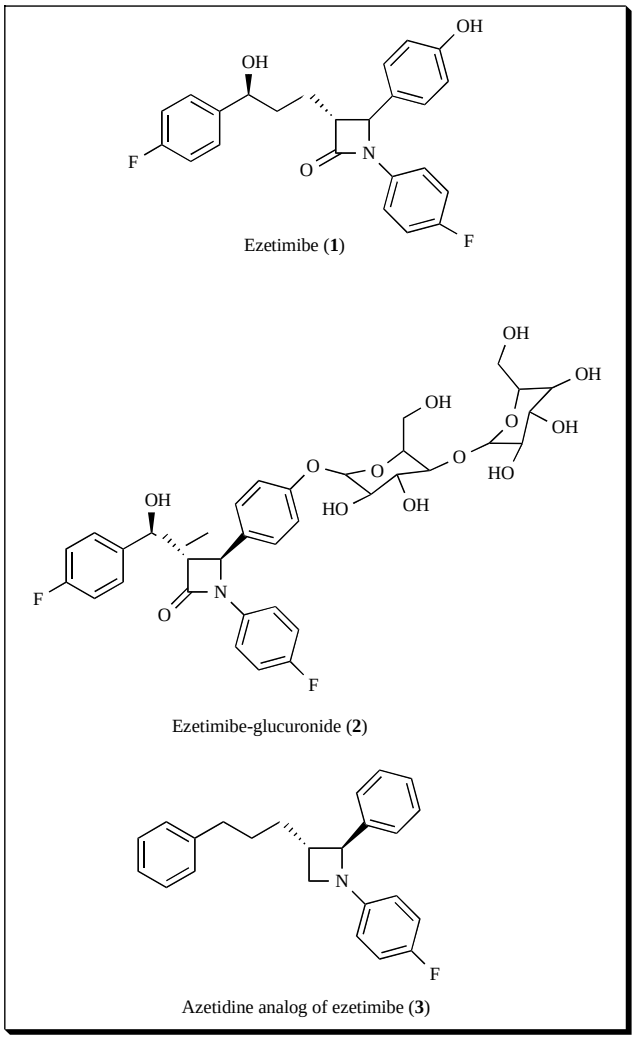
Apoprotein (MW (Da))	Lipoprotein Association	Function and Comments
apoA-I (29,016)	Chylomicrons, HDL	Major protein of HDL, activates lecithin: cholesterol acyltransferase (LCAT)
apoA-II (17,400)	Chylomicrons, HDL	Primarily in HDL, enhances hepatic lipase activity
apoA-IV (46,000)	Chylomicrons and HDL	Present in triacylglycerol rich lipoproteins
apoB-48 (241,000)	Chylomicrons	Exclusively found in chylomicrons, derived from apoB-100 gene by RNA editing in intestinal epithelium; lacks the LDL receptor-binding domain of apoB-100
apoB-100 (513,000)	VLDL, IDL and LDL	Major protein of LDL, binds to LDL receptor; one of the longest known proteins in humans
apoC-I (7,600)	Chylomicrons, VLDL, IDL and HDL	May also activate LCAT
apoC-II (8, 916)	Chylomicrons, VLDL, IDL and HDL	Activates lipoprotein lipase
apoC-III (8,750)	Chylomicrons, VLDL, IDL and HDL	Inhibits lipoprotein lipase
apoD (33,000)	HDL	Closely associated with LCAT activation
Cholesterol Ester Transfer Protein, (CETP)	HDL	Exclusively associated with HDL & cholesteryl ester transfers
apoE (34000) (at least 3 alleles [E_2, E_3, E_4] each of which have multiple isoforms)	Chylomicron remnants, VLDL, IDL and HDL	Binds to LDL receptor, $apoE_{e\text{-}4}$ allele amplification associated with the late-onset of Alzheimer's disease
apo(a) –(3,00,000-8.00,000) at least 19 different alleles.	LDL	Disulfide bonded to apoB-100, forms a complex with LDL identified as lipoprotein(a) or Lp(a); strongly resembles plasminogen; may deliver cholesterol to sites of vascular injury. High risk association with premature coronary artery disease and stroke

One of the possible options for lipid control which has attracted the attention of investigators is the intervention of cholesterol absorption in the small intestine. The precise mechanism of cholesterol absorption is not known. Recently, the Niemann-Pick C1 Like 1 (NPC1L1) polytopic transmembrane protein has been identified as a putative cholesterol transporter and plays critical role in cholesterol absorption, and thus is the site of action of a new lipid lowering drug ezetimibe (**1**), in intestinal cells. Blocking NPC1L1 endocytosis dramatically decreases cholesterol internalization, indicating that NPC1L1 mediates cholesterol uptake *via* its vesicular endocytosis [22]. Ezetimibe(**1**) chemically, (3R, 4S)-1-(4-flurophenyl)-3-[(3S)-3-(4-flurophenyl)-3-hydroxypropyl]-4-(4-hydroxypropyl)-2-azetidinone, prevents NPC1-L1 from incorporating into clathrin-coated vesicles and thus inhibits cholesterol uptake [23]. It is suggested that cholesterol is internalized into cells with NPC1L1 through clathrin/AP2-mediated endocytosis and ezetimibe inhibits cholesterol absorption by blocking the internalization of NPC1L1 [22]. The NPC1L1 protein is also expressed in liver cells and probably plays a significant role in the development of diet-induced fatty liver [24].

It is estimated that ezetimibe lowers diet cholesterol absorption in the intestine by over 50% [25]. Ezetimibe-glucuronide (**2)** is the pharmacologically active metabolite of the drug with bioavailability of 80% and its maximal concentration is reached within 1-2 hours after administration [26].

More recently, the development of an *in vitro* binding assay using a number of ezetimibe analogues further strongly indicated NPC1L1 to be critically involved. These ezetimibe analogues were shown to bind to the brush border membranes and to cells expressing NPC1L1 with virtually identical binding affinities. In contrast no binding was observed to the brush border membrane of NPC1L1 knock-out mice [27]. In a recent intriguing finding, it was alternatively suggested that NPC1L1 is not actually located on the brush border membrane, but is found inside the enterocytes. This latter finding highlights the complexity of intestinal cholesterol absorption, indicating it to be a multistep process that presumably involves a number of proteins. These new inhibitors can be divided into two general compounds classes [28]:

1. Ezetimibe derivatives in which the phenol is conjugated to various carbohydrates or derivatized with simple substituents, *i.e.*, compound (**2)**
2. Analogues of ezetimibe with the β-lactam of ezetimibe replaced by the corresponding azetidine. *i.e.,* compound (**3)**

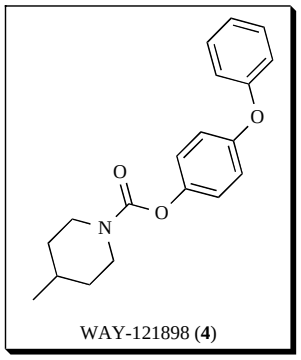


An altogether different structure, *viz*., compound WAY-121898 (**4)** reportedly inhibits cholesterol ester hydrolysis [29] with IC_{50} value of 0.2 M and reduces absorption of a single dose of cholesterol in normal diet fed rats with an ED_{50} of 10 mg/kg [30].

5.2. Peroxisome Proliferation Activated Receptor (PPAR) Agonists

PPAR are nuclear receptors that are ligand dependent nuclear transcription factors implicated in the regulation of lipids and glucose metabolism, morphogenesis, cell growth, cell differentiation and homeostasis. PPAR is a subfamily of the 48 member nuclear receptors super family [31] and regulates gene expression in response to ligand binding [32, 33].

Three mammalian PPARs have been identified and are termed as PPAR-α, -γ & -δ. PPAR's regulate expression of target genes by binding to DNA sequences, termed as PPAR response elements. Studies have shown that PPAR agonists can serve as very good candidates for antihyperlipidemic, as well as, antihyperglycemic activity. The PPAR-γ receptor subtype is predominantly expressed in adipose tissue and plays a pivotal role in adipocyte differentiation, suggesting PPAR-γ as an important component in the adipogenic signaling cascade and in lipid storage and utilization. The human PPAR-γ gene structure has been characterized and its two isoforms with a common ligand binding domain, PPAR-γ_1 and PPAR-γ_2 have been identified.

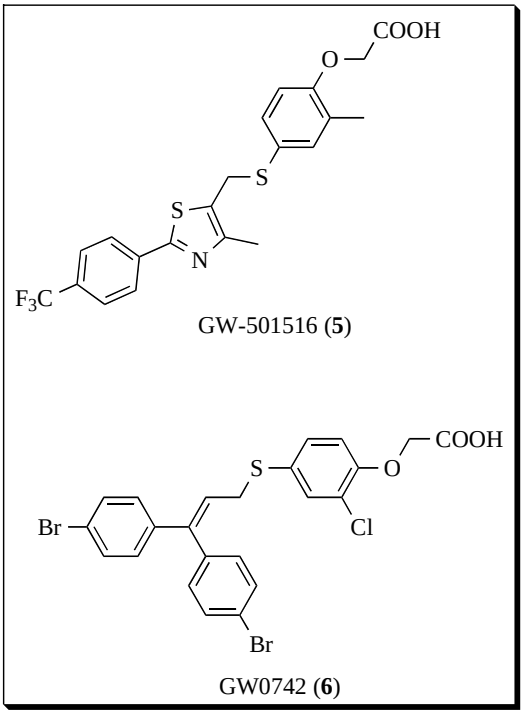


PPAR-α activation enhances free fatty acids oxidation, controls expression of multiple genes regulating lipoprotein concentraton and anti-inflammatory effects [34].

Li *et al.*, [35] found PPAR-α and PPAR-γ ligands protective against atherosclerosis and inhibit macrophage foam cells formation. PPAR-α activated by polyunsaturated fatty acids and fibrates, is implicated in regulation of lipid metabolism, lipoprotein synthesis and inflammatory response in liver and other tissues. PPAR-α is highly expressed in tissues with high fatty acid oxidation (like liver, kidney and heart muscle), in which it controls a comprehensive set of genes that regulate most aspects of lipid catabolism. Like several other nuclear hormone receptors, it heterodimerizes with RXR-α to form a transcriptionally competent complex. In addition, PPAR-α is expressed in vascular endothelial cells, smooth muscle cells, monocyte/macrophages and T lymphocytes. PPAR-α activation increases HDL cholesterol synthesis, stimulates "reverse" cholesterol transport and reduces triglyceride absorption. PPAR-γ plays important roles in the regulation of proliferation and differentiation of several cell types, including adipose cells. It has the ability to bind to a variety of small lipophilic compounds derived from both metabolism and nutrition. These ligands, in turn, determine cofactor recruitment to PPAR-γ, regulating the transcription of genes in a variety of complex metabolic pathways.

The fact that PPAR-δ plays an important role in atherosclerosis has been reported by several studies in different mouse models with different approaches. Treatment of obese animals by specific PPAR-δ agonists like GW501516 (**5**) and GW0742 (**6)** [36] results in normalization of metabolic parameters and reduction of adiposity. PPAR -δ appears to be implicated in the regulation of fatty acid burning capacities of skeletal muscles and adipose tissue by controlling the expression of genes involved in fatty acid uptake, β-oxidation and energy uncoupling. PPAR -δ is also implicated in inducing the adaptive metabolic response of skeletal mus -cles to endurance exercise by controlling the number of oxidative myofibers and also enhancing fatty acid catabolism in muscular tissues. Moreover, recent studies have revealed that ligand activation of these receptors is associated with improved insulin sensitivity and elevated HDL levels thus demonstrating promising potential for targeting PPAR –δ in the treatment of obesity, dyslipidemias and type 2 diabetes.

Lee *et al*., [37] have observed PPAR- δ to be proatherogenic as well as proinflammatory by bone marrow transplantation studies in $LDLR^{-/-}$ mice. The agonist GW501516 (**5**) has besn demonstated to have clear antiatherosclerotic property in $apoE^{-/-}$ mice. Following this loss-of-function approach, two independent studies examined the effect of the PPAR-δ agonist GW0742 (**6**) on atherogenesis in high fat and cholesterol-fed $LDLR^{-/-}$ mice and yielded divergent results. In the first study, Li *et al*., found that GW 0742 (**6**) decreased gene expression of proinflammatory cytokines and adhesion molecules within atherosclerotic lesions, but failed to alter the progression of atherosclerosis after 14 weeks of treatment (5 mg/ kg/ day). In another study, Graham *et al.*, used female $LDLR^{-/-}$ mice fed with a diet that induced moderate levels of hypercholesterolemia and observed that GW0742 reduced the lesion size at a higher dose (60mg/ kg/ day) after 10 weeks of treatment [38]. Discrepancy between these two studies may be caused by differences in the levels of hypercholesterolemia and different drug doses used. However, the anti-inflammatory effect was generally consistent in both studies, regardless the different effects on the lesion sizes. It is likely that the anti-inflammatory properties of the PPAR-δ agonists on the vessel wall *per se* are not sufficient to attenuate the progression of atherosclerotic lesions, if it is not corroborated by an efficient improvement of metabolic abnormalities



Barish *et al*., [39] have shown that GW501516 (**5**) significantly reduced atherosclerotic lesions with an increase in HDL levels and a reduced expression of chemokines in the aorta and in macrophages. Furthermore, in a model of angiotensin-II accelerated atherosclerosis ($LDLR^{-/-}$ mice), Takata *et al*., [40] confirmed the atheroprotective effect of GW0742 (**6**). After 4 weeks of treatment, GW0742 (**6**) at two doses (1 and 10mg/kg/day) significantly inhibited the angiotensin-II induction of atherosclerosis, without altering blood pressure. This beneficial effect was likely to be mediated *via* the potent anti-inflammatory property, since GW0742 (**6**) increased vascular expression of Bcl-6 (B-cell CLL/ lymphoma 6), the regulators of G protein-coupled signaling (RGS4 and 5) in the artery and suppressed angiotensin- II induced activation of p38 and ERK (Extracellular Signal-Regulated Kinase) in macrophages. However, the metabolic effect of GW0742 (**6**) may also have contributed to the atheroprotective outcome, because GW0742 (**6**) significantly reduced plasma levels of insulin, glucose, leptin, and decreased triglycerides [40]. Overall, studies in mouse models suggest that PPAR-δ may have an attractive therapeutic target for the treatment of atherosclerosis.

To further explore the mechanism of the PPAR actions in atherogenesis Li *et al*., [35] have developed a novel approach for evaluating macrophage foam-cell formation *in vivo*. This approach involves the adoptive transfer of peritoneal macrophages from various PPAR null mice into the peritoneal cavity of mice fed with a cholesterol rich diet. This innovative assay provides a new model for macrophage lipid uptake although; further validation is needed to clarify whether the assay faithfully reflects the actions of artery wall macrophages during atherogenesis. Nevertheless, Takero *et al.*, [41] have rerported that PPAR-α and PPAR-γ ligands inhibit lipid accumulation in a receptor- dependent manner, pointing to direct protective mechanisms within the macrophage. PPAR-γ ligands also markedly reduce cholesterol esterification in macrophages. This newly appreciated activity may be a significant contributor to the atheroprotective effects of PPAR-γ agonists. PPAR-γ ligand–treated mice have shown increased cholesterol efflux to HDL, consistent with this mechanism. The study has provided new insights into pathways regulating macrophage lipid accumulation and rounds out the family picture of PPARs in atherosclerosis. Both PPAR-α and PPAR-γ agonist are reported to protect against atherosclerosis and inhibit macrophage foam-cell formation. Finally, this work has emphasized the fact that PPAR agonists mostly exert their anti-atherosclerotic properties by multiple mechanisms, including improving systemic lipid levels, improving insulin resistance, and inhibiting the accumulation of macrophage foam cells. Cholesterol accumulation in macrophages located in the endothelium is a crucial step in the formation of atherosclerosis. PPAR-γ activation is necessary for the efflux of cholesterol from macrophage foam cells. Cholesterol taken up by HDL particles containing apolipoportein A-1 is transported to the liver to be disposed of as bile acids. PPAR-α agonists, on the other hand, speed up the transfer of cholesterol from macrophages to particles containing apolipoproteins A-1. All PPARs bind to a direct repeat of two hexanucleotides, spaced by one or two nucleotides (the DR1 or DR2 motif) as heterodimers with the retinoid X receptor (RXR), and activate several target genes [42].

To summarize, PPAR–δ can be a good target for the treatment of obesity, dyslipidemia and type 2 diabetes, while PPAR-α and PPAR-γ stimulations play complementary roles in the prevention of atherosclerosis. Thus, PPAR- α, γ and δ can be good targets for atherosclerotic therapy.

5.3. Acyl-CoA Cholesterol Acyl Transferase (ACAT)

Acyl Co-A transferase (ACAT) is an endoplasmic reticulum-bound intracellular enzyme that catalyzes the formation of cholesteryl esters from cholesterol and long chain fatty acyl CoA in a wide variety of cells [43]. Two ACAT isozymes have been identified till date. ACAT-1, an isozyme essential for intracellular storage of cholesteryl esters, is expressed ubiquitously in various human tissues and cells, such as the adrenal glands, sebaceous glands, various types of macrophages, liver and intestine. ACAT-2 is expressed exclusively in the small intestine and liver. ACAT-2 is no doubt a major ACAT isozyme in small intestine and plays a crucial role in chylomicron assembly and cholesterol absorption [44]. In human liver, both the ACAT isozymes are expressed and may provide cholesteryl esters for VLDL. However, contribution of each isozyme to hepatic ACAT activity is still controversial. Atherosclerotic lesions are characterized by accumulation of macrophage-derived foam cells. These macrophage derived foam cells express high levels of ACAT-1 protein. Expression of ACAT-1 increases during differentiation of human monocytes into macrophages, which is a prerequisite for formation of macrophage derived foam cells [45].

Thus, ACAT inhibitors are thought to have two different pharmacological actions: suppression of cholesterol absorption in the intestine that leads to reduction of plasma cholesterol level, and suppression of foam cell formation in the arterial walls. The direct suppression of foam cell formation in atherosclerotic lesions, which is independent of the cholesterol lowering effect, is a more attractive aspect of ACAT inhibitors. As ACAT-1 is the dominant isoenzyme expressed in macrophages, ACAT-1 is regarded as a target molecule for the ACAT inhibitors in the arterial walls. However, selective disruption of macrophage ACAT-1 by bone marrow transplantation from ACAT-1-null mice to LDL receptor null mice results in exacerbation of diet-induced atherosclerosis.

The drugs avasimibe (**7**) and eflucimibe (**8**) act by inhibiting ACAT in the arterial wall thus slowing the development of atherosclerosis by several possible mechanisms [46]. The following compounds, **9-12**, have been shown to inhibit ACAT *in vitro* [47-50]. The molecule KF-17828 (**10**) has been reported to accelerate the regression of hypercholesterolemia in cholesterol fed hamsters implying its systemic effect to be more profound than simple withdrawal of dietary cholesterol. Some of the most effective members of this class, RP-70676 (**13**) and compound **14** are currently in clinical development [51, 52].

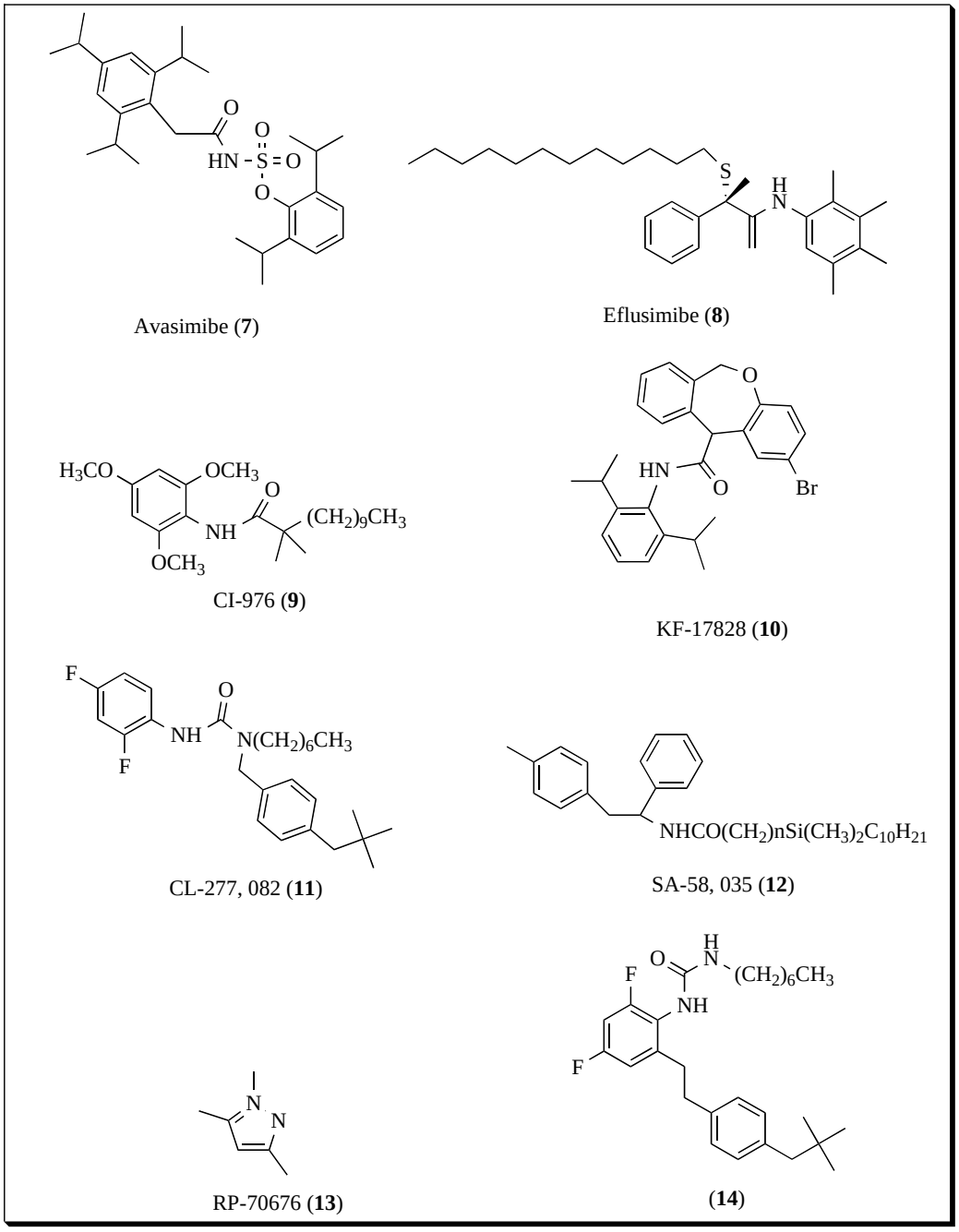
ACAT inhibitors have been shown to reduce cholesteryl ester content in various cell cultures [50, 53]. In macrophages and cholesteryl ester-rich hepatoma cell lines, the cholesteryl ester-reducing effect of ACAT inhibitors is generally attributed not only to direct inhibition of ACAT, but also to their ability to increase cholesterol efflux to various cholesterol acceptors present in the growth medium [54-56]. In 1991, Bocan *et al.*, [57] showed that feeding low-dose ACAT inhibitor CL-976 (**9**) to cholesterol-fed rabbits reduced the size of foam cell lesions without affecting plasma cholesterol levels. This study suggests that at low dose, the primary action of the ACAT inhibitor occurs at the foam cell and not at the liver. It also provides the first *in vivo* evidence that ACAT inhibitors may possess an anti-atherosclerosis effect without significantly affecting total plasma cholesterol levels. However, other laboratories using ACAT-1 gene knockout mouse models obtained disappointing results. Accad *et al.*, [58] and Yagyu *et al.*, [59] have reported that the double-knockout ($ACAT^{-/-}$ with apo $E^{-/-}$ or $LDLR^{-/-}$) mice developed extensive deposition of free cholesterol in the skin and brain [58, 59]. Fazio *et al.*, [60] have reported that LDL receptor deficient ($LDLR^{-/-}$) mice, reconstituted with $ACAT\text{-}1^{-/-}$donor bone marrow, showed accumulation of free cholesterol in their artery wall, and thus developed larger atherosclerotic lesions than the control LDLR-/- mice. The authors suggest that inhibition of ACAT1 may promote lesion development. However, data from knockout mice models may not always be relevant to human pharmaceutical applications. $ACAT\text{-}1^{-/-}$ mice show a complete absence of ACAT1 enzyme, a condition that does not occur with pharmaceutical interventions on ACAT1 in animals or in human patients. Therefore, the knockout mice model is probably only appropriate for demonstrating the effects of drug overdoses that may cause detrimental effects in animals or in humans. To investigate the efficacy of partial ACAT inhibition, Kusunoki *et al.*, [61] have suggested that partial inhibition of ACAT could be an effective therapeutic treatment for atherosclerosis through study on apoE knockout mice with low dose ACAT inhibitor [57]. One early concern in developing ACAT inhibitors was adrenal toxicity [62]. Another major concern for using the ACAT inhibitor is cellular toxicity induced by free cholesterol loading [63, 64]. The *in vivo* significance of these findings is unclear. However, these findings do raise concerns for using ACAT inhibitors for treating atherosclerotic lesions under conditions where cellular sterol efflux becomes severely hampered.

There are currently several ACAT inhibitors being tested in clinical trials. Sahi *et al.*, [65] have shown that the ACAT inhibitor avasimibe (**7**) induces the cytochrome CYP3A4 in human hepatocytes, thus promoting the hepatic inactivation of drugs concurrently administered. If combination therapy is to be tested, care must be exercised to avoid drug-drug interactions.

Thus, inhibition of ACAT appears to be one of the strategies, which is different from the effect of statins, which works mainly by reducing plasma cholesterol. The ACAT inhibitors may work by directly reducing the size of the lipid-rich core in the atherosclerotic plaques, thus stabilizing the lesion against plaque rupture.

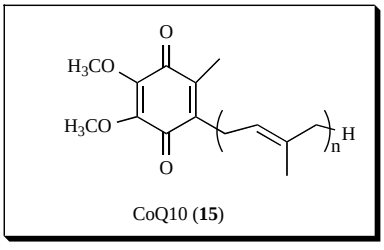
5.4. Coenzyme Q10

Coenzyme Q10 (CoQ10) (**15**) is a fat-soluble vitamin like antioxidant found in virtually all cell membranes, hence its alternative name is "ubiquinone". By the mid-1960s, Japanese researchers recognized that CoQ10 is particularly concentrated in the myocardium, or heart muscle. Its role in the heart makes sense: the heart, one of the body's most energetic organs, beats approximately 100,000 times a day and 36 million times a year, and depends on CoQ10 for "bioenergetics." In the early 1980s, the first study on CoQ10 was



conducted for its usefulness in the treatment of cardiomyopathy, a form of progressive heart failure [66].

The biosynthesis of CoQ10 from the amino acid tyrosine is a 17 steps process requiring, various B complex vitamins and several trace elements. Coenzyme Q10 is also an essential component of the mitochondria playing a critical role in the formation of ATP [67-69], the body's fundamental energy unit, from carbohydrate and fatty acid metabolism. CoQ10 is the coenzyme for at least three mitochondrial enzymes (complexes I, II and III), as well as, enzymes in other parts of the cell. Mitochondrial enzymes of the oxidative phosphorylation pathway are essential for the production of the high-energy phosphate, adenosine triphosphate (ATP), upon which all cellular functions depend [70]. Coenzyme Q10 also appears to increase adenosine triphosphate levels by preventing the loss of the adenine nucleotide pool from cardiac cells. It appears that coenzyme Q10 might be involved in maintaining the proper pH of lysosomes, which are a digestive component of cells, as well.



The antioxidant activity of vitamin E requires the CoQ10 to be available, to which vitamin E will pass on the unpaired electron (free radical) that it has scavenged. Additionally, coenzyme Q10 has demonstrated activity in preventing lipid peroxidation as an antioxidant scavenger and an indirect stabilizer of calcium channels to decrease calcium overload. For some time there has been a view that the muscle pains associated with statins may be reversed by taking coenzyme Q10. The argument is that statins inhibit the biosynthesis of both cholesterol and ubiquinone with resultant lowering of cholesterol and ubiquinone in blood and muscles. As ubiquinone is involved in electron transport and ATP formation, this leads to fatigue and muscle pain.

Unfortunately, HMG-CoA reductase inhibitors used to treat elevated blood cholesterol levels by blocking cholesterol biosynthesis also block CoQ10 synthesis [71]. The resultant lowering of blood CoQ10 level is due to the partially shared biosynthetic pathway of CoQ10 and cholesterol. This has been observed in many patients with heart failure and has significant harmful effects which ofcourse can be negated by oral CoQ10 supplementation. Most of the reduction in circulating coenzyme Q10 is related to lower levels of LDL-cholesterol and some studies suggest that absolute changes are less relevant than the coenzyme Q10 / cholesterol ratio.

CoQ10 is carried in the blood with LDL and serves to diminish the oxidant of LDL-C settings of oxidative stress [72]. Thus, CoQ10 is beneficial in treating and preventing CVDs (cardiovascular diseases) and conditions such as high blood pressure [73], atherosclerosis [74], angina [75] and CHF (congestive heart failure) [76]. Thus, CoQ10 can be a good target, and drugs helping in elevating its levels can be beneficial.

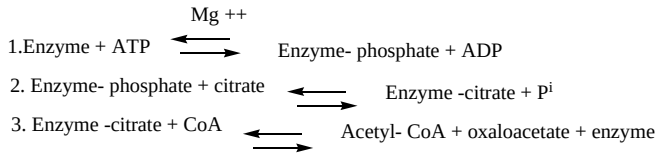
5.5. ATP Citrate Lyase

ATP citrate lyase (ACL) is the primary group of enzymes responsible for the synthesis of cytosolic acetyl CoA in many tissues. As the name suggests, ACL catalyses the cleavage of citrate into acetyl CoA and oxaloacetate [77-79], with a concomitant hydrolysis of ATP to ADP and phosphate. ACL is the group of cytosolic enzymes found in large variety of animal tissues and its activity is particularly high in lipogenic tissues such as liver [80]. It is proven that ACL is located in the cytosol, and is not detectable in the plastids, mitochondria or peroxisomes.

The enzyme is a tetramer (M.wt (*c.a.)* 440,000) of four apparently identical subunits. It is positioned upstream of HMG CoA reductase in the mammalian cholesterol biosynthetic pathway and supplies acetyl units for both cholesterol and fatty acid synthesis [81]. Acetyl-CoA is an intermediate of both primary and secondary metabolism. Acetyl-CoA requiring metabolism occurs in a number of subcellular compartments. Because acetyl-CoA cannot readily cross membranes, compartmentalized biosynthesis is required. Cytosolic acetyl-CoA can undergo carboxylation or condensation, which generates malonyl-CoA or acetoacetyl-CoA, respectively. Products from the carboxylation pathway contribute to the elongation of fatty acids and the biosynthesis of a variety of other phytochemicals, including flavonoids and malonic acid. The condensation pathway on the other hand leads to the biosynthesis of HMG-CoA-derived isoprenoids, including sterols and brassinosteroids.

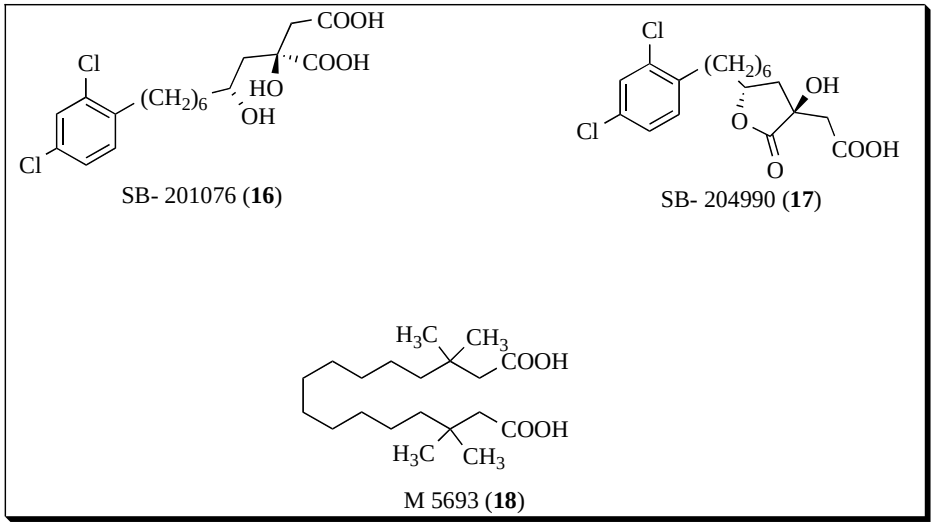
ACL is an essential enzyme of the reductive tricarboxylic acid (RTCA) pathway of CO_2 assimilation. The RTCA pathway occurs in several groups of autotrophic prokaryotes, including the green sulfur bacteria. ACL catalyzes the coenzyme A dependent and Mg-ATP-dependent cleavage of citrate into oxaloacetate and acetyl-CoA, a key step in the RTCA pathway [82].

The reaction mechanism of ACL enzyme has been investigated by studies on rat liver preparation, using radio labeled ^{14}C-ATP and ^{32}P-ATP, of which the later seems to be important along with magnesium ions to yield ultimately acetyl-CoA and oxaloacetate [83]. The overall reaction mechanism is summarized as follows.



Inhibition of ACL could be anticipated to; (1) decrease the plasma LDL cholesterol levels in similar manner to HMG CoA reductase inhibition, by decreasing cholesterol synthesis and upregulating LDL receptor activity and (2) decrease the plasma triglyceride levels as a consequence of reduction in synthesis of fatty acids leading to ultimate lowering of plasma VLDL. As a precursor for LDL, reduction in VLDL may also contribute further to plasma LDL lowering. Cytosolic level of malonyl CoA is also expected to decrease after inhibition of ACL. As malonyl CoA is known to regulate the entry of acyl moieties into mitochondrial β-oxidation spiral, inhibition of ACL is expected to channelize hepatic free fatty acids into β-oxidation, thereby further decreasing the pool of fatty acids available for triglyceride and VLDL synthesis. ACL catalyses reversible retro-Claisen reaction utilizing ATP citrate and CoASH as substrates to yield ADP, inorganic phosphate (P^i) oxaloacetate and acetyl CoA. This differs from the originally accepted mechanism for catalysis which has invoked the necessity for active site thiol nucleophile to allow the formation of covalently bound citral enzyme adduct. Studies with (-)hydroxycitrate, a potent ATP citrate lyase inhibitor have demonstrated, that inhibition of this enzyme leads to decrease in the synthesis of both cholesterol and fatty acids [84-88] and an increase in the LDL receptor activity [89]. Further, a decrease in plasma cholesterol and plasma triglyceride levels was found in rats treated with (-) hydroxycitrate, suggesting a potential utility of ATP citrate lyase inhibitors as hypolipidemic agents [88]. Pearce *et al.,* [90] have identified a series of potent inhibitors of ATP citrate lyase such as SB-201076 (**16**) having a k_i of 1μM for the human enzyme. Also its prodrug lactone SB-204990 (**17**), inhibited cholesterol and fatty acids synthesis in Hep G2 cells (dose related inhibition up to 91% and 82%, respectively) and in rats (76% and 39 %, respectively).

A series of 2-substituted butanedioic acids has been designed and synthesized as inhibitiors of the enzyme [91].



Another compound, MEDICA 16 (M 5693) (**18)**, reduced plasma lipids dramatically [92, 93].

Thus, ACL can be considered as a good target for controlling the plasma lipid levels, especially the LDL, VLDL and TG levels. Inhibitors of ACL can be designed for this purpose to act as novel antihyperlipaemic agents.

5.6. Enhancement of HDL Levels

Elevated levels of some HDL particles appear to be correlated to a decrease in the number of sites of stenosis on the coronary arteries as seen during some human angiographical studies [94]. HDL may protect against the progression of atherosclerosis through several mechanisms [95]. *In vitro* study has shown that HDL is capable of removing cholesterol from cells. The antiatherogenic property of HDL may lie in its ability to deplete tissues of excess free cholesterol and essentially leading to the delivery of this cholesterol to the liver [96]. In addition HDL may serve as a reservoir in the circulation for apoproteins necessary for the rapid metabolism of triglycerides rich lipoproteins (TRL). Accordingly, agents that increase serum HDL concentration should be of utility as antiatherosclerotic agents that may be useful in the treatment of dyslipoproeinema and related coronary heart diseases.

The HDL fraction is responsible for reverse cholesterol transport and is therefore antiatherogenic in nature and it is proposed that a reduction of plasma-HDL concentration may accelerate the development of atherosclerosis, and hence ischaemic heart-disease, by impairing the clearance of cholesterol from the arterial wall [97, 98]. After a fat load, in normolipidemic subjects HDL concentration usually remains unchanged [99-102] or decreases slightly [103]. However, HDL composition changes profoundly as lipids and apolipoproteins are exchanged between HDL and triglyceride particles. This exchange enriches the HDL fraction with triglyceride and decreases its apoA-I concentration [103]. Individuals with high-level postprandial lipemia exhibited a decrease of the large HDL2 subfraction and an increase in the HDL3 subfraction, but little or no change occurred in subjects with lower degrees of postprandial lipemia and high fasting concentrations of HDL2 [104].

Plasma HDL subpopulations arise atleast partly through lipid transfer reactions mediated by transfer proteins and enzymes. These include cholesteryl ester transfer protein (CETP), lecithin: cholesteryl acyltransferase (LCAT), hepatic lipase and phospholipid transfer proteins (PLTP) [105].

CETP catalyzes the exchange of neutral lipids, particularly TG and cholesteryl esters, amongst all major lipoprotein classes. In the postprandial state, in normolipidemic subjects, cholesteryl ester transfer has been shown to remain unchanged [106, 107] or increase [98, 108]. The latter arises partly from an increase in the concentration of acceptor particles, from redistribution of CETP, from increase in CETP activity and probably also from an increase in CETP mass [109]. LCAT is essential in maintaining the unesterified cholesterol concentration gradient between cell membranes and plasma HDL in reverse cholesterol transport. This active TG/cholesteryl ester exchange may initiate a remodeling of HDL, eventually resulting in the formation of pre-β-HDL which is the initial acceptor of free cholesterol released from the cell surface [98]. Two possible functions of phospholipid transfer protein (PLTP) in lipoprotein metabolism have been proposed. First, PLTP may act in the transfer of surface fragments formed during lypolysis of TRL to HDL [110]. Second, PLTP may act in HDL conversion [111, 112].

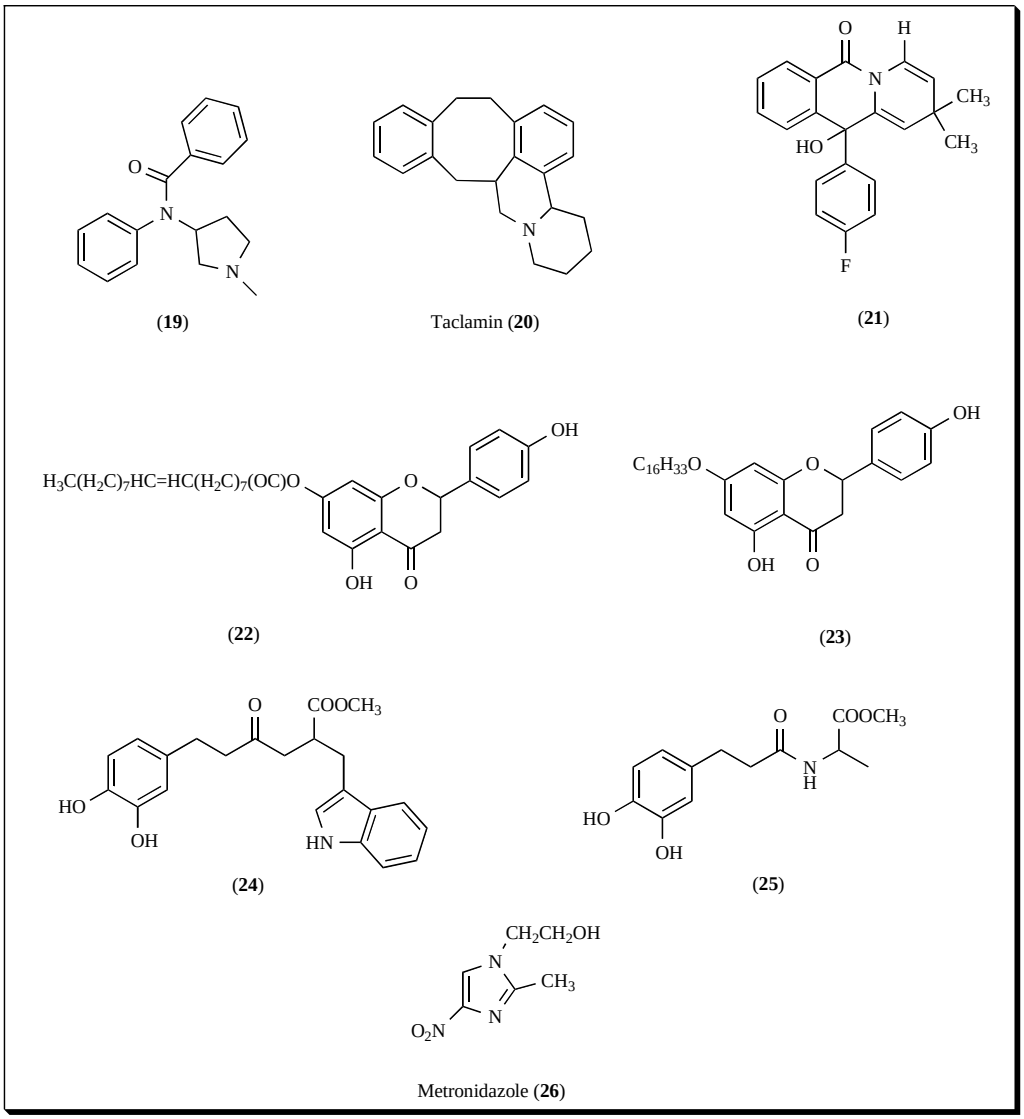
Of the compounds that are reported to enhance HDL properties, the main are sulfur-containing classes of compounds such as thiohydantoin, thiouracils, thiosemicarbazone and sulfonyl imidazolidinediones. The benzamide derivative, (**19)** and taclamin (**20**) are amongst the non-sulfur containing hits. Based on the common structural elements of the non-sulfur containing compounds, a pharmacophore has been generated and a 3D search of the database has led to the identification of a series of compounds with a novel tricyclic imidazoisoquinolone structure, prototype of which is the compound (**21)**. Several derivatives of this series have exhibited potent HDL enhancing activity in various animal models [113].

Other nonsulfur containing HDL enhancing compounds effective as antiatherogenic agents are the citrus flavonoids namely, naringenin derivatives (**22** and **23**). The aglycones of these compounds have been found to inhibit the formation of aortic atherosclerotic lesions in rabbits fed with high diet cholesterol [114]. Naringenin derivatives, ester (**22**) and ether (**23**) significantly inhibit the formation of aortic fatty streaks in high cholesterol fed rabbits with already existing atherosclerotic lesions [115]. A series of 3,4-dihydroxyhydrocinnamides (**24)** and (**25)** have exhibited antiatherogenic activity by inhibiting the formation of fatty streaks in high cholesterol fed rabbits [116].

Imidazole derivatives like metronidazole (**26**) act as antiatherogenetic in a similar manner as the imidazoquinolones, which have already been found to increase HDL cholesterol in animals [117].

5.7. Microsomal Triglyceride Transfer Protein (MTP)

Microsomal triglyceride transfer protein (MTP) is required for the secretion of apo B–containing lipoproteins from hepatocytes, as well as, the absorptive enterocytes of the intestine [118, 119]. MTP is also found in other tissues such as the myocardium [120, 121], yolk sac [122], and kidney [123], where apo B–containing lipoproteins are synthesized. MTP is located within the lumen of the endoplasmic reticulum (ER), where it is assumed to transfer lipids during the assembly of lipoproteins. In the *in vitro* systems, MTP transfers both neutral and polar lipids between liposomal membranes, but lipid transfer is most efficient with neutral lipids, (triglycerides and cholesterol esters). The MTP holoprotein is a heterodimer consisting of a 55-kDa multifunctional protein (protein disulfide isomerase) and a unique 97-kDa subunit. ApoB is a large (4563 amino acids) amphipathic protein which serves as the major structural protein in VLDL. Two major forms of apoB are produced through a unique m-RNA editing step. In humans, apoB-100 is made exclusively by the liver, whereas apoB-48 is made in the intestine [124]. The importance of MTP for the secretion of apoB–containing lipoproteins was revealed in 1992, when Wetterau *et al.,* [125] reported that both MTP and its activity were absent in duodenal tissue of humans suffering with a β lipoproteinemia.



MTP is widely believed to play an important role in this first "apoB lipidation" step [126]. In a second step, the lipidated apo B molecule is thought to acquire the bulk of its core lipids by fusing with a large, VLDL-sized, apoB–free triglyceride particle or a "second- step" particle [127, 128]. The existence of these second step triglyceride particles within a special compartment of the smooth ER has been supported by 2 different electron microscopic studies [129, 130]. Biochemical studies of VLDL assembly have also supported the concept that the bulk of neutral lipids are added to apo B in a second step after its translation is complete [118, 130]. The role of MTP, if any in the formation of second-step lipid particles is unclear [131, 132]. Using an MTP inhibitor drug and complex metabolic labeling protocols, one group of researchers has concluded that MTP has little or no role in the addition of the bulk of lipids to nascent apo B-48–containing lipoproteins [133]. However, another group of researchers using a similar approach and the same MTP inhibitor, has reached the opposite conclusion that MTP is essential for adding most of the triglycerides to apo B–containing VLDL during the second step of lipoprotein assembly [134]. MTP, a resident protein in the lumen of the endoplasmic reticulum, facilitates the transfer of triglycerides from their site of synthesis in the endoplasmic reticulum into the lumen during the assembly of VLDL [135]. It is believed that blocking MTP will not only reduce plasma levels of total LDL cholesterol (LDL-C), but also those of VLDL and triglycerides by affecting the packaging and secretion of VLDL and chylomicrons [136].

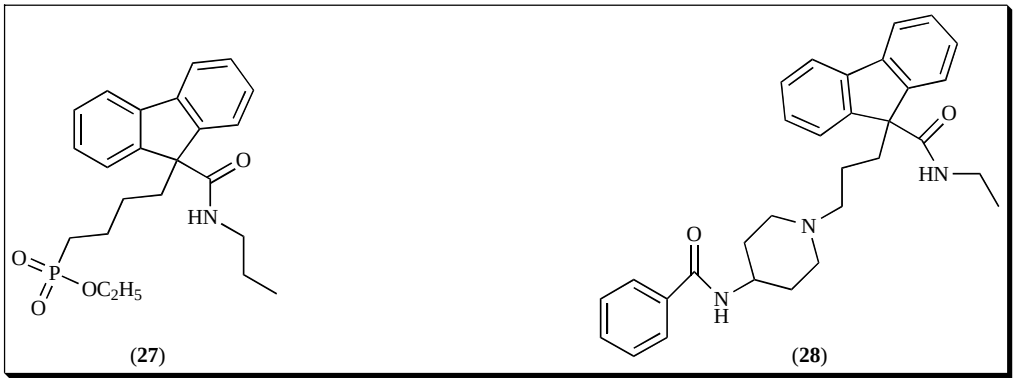
Certain alkyl phosphonates (**27**) have been synthesized and evaluated to exhibit potent MTP inhibition, both *in vitro* and *in vivo* [137].

A series of MTP inhibitors (**28**) has been reported to normalize the plasma lipoprotein levels in Watanable heritable hyperlipidemic rabbits [138].

5.8. Cholesterol Ester Transfer Protein (CETP)

CETP is secreted mainly from the liver and then it circulates in plasma, bound mainly to HDL as a hydrophobic glycoprotein [139]. It promotes the redistribution of cholesterylesters, triglycerides, and to a lesser extent, phospholipids between plasma lipoproteins. CETP transfers lipids from one lipoprotein particle to another, in a process that results in equilibration of lipids between lipoprotein fractions [140]. Most of the cholesterylesters in plasma originate in the HDL by these actions catalyzed by LCAT and majority of the triglycerides enter the plasma as components of chylomicrons and LDL as well as, triglyceride-rich lipoproteins (TRLs). The overall effect of CETP is a net mass transfer of cholesteryl esters from HDLs to TRLs and that of LDLs and triglycerides from TRLs to LDLs and HDLs. Thus, CETP-mediated transfers from HDL to VLDL and LDL provide a potential indirect pathway by which HDL cholesteryl esters can be delivered to the liver [141]. Several species, including mice and rats, are naturally deficient in CETP. Introduction of the human CETP gene into mice results in a dose-related reduction in plasma HDL levels and a small increase in VLDL and LDL cholesterol and apoB levels [142, 143]. In fact, in studies of bile salts and cholesterol-fed, C57-B16 mice, the introduction and expression of the similar CETP genes in them has resulted in enhanced formation of fatty streak lesions compared to the nonexpressing controls [144]. It has been concluded that the enhancement of these fatty lessions development by CETP is secondary to a redistribution of cholesterol from HDLs to the VLDL/LDL atherosclerosis. These results again support that CETP is proatherogenic. Rabbits also have naturally high levels of CETP. Furthermore, it has been demonstrated in several rabbit models of atherosclerosis, that inhibiting CETP results in a marked reduction in atherosclerosis. In cholesterol-fed rabbits, the inhibition of CETP by injection of antisense oligodeoxynucleotides (ODNs) has resulted in a reduction in CETP m-RNA and mass in the liver, a reduction in plasma total cholesterol and an increased concentration of HDL cholesterol. A newly developed chemical inhibitor of CETP has been used in another recent study [145] of cholesterol-fed rabbits, which has reduced the CETP activity by 90%, almost doubled the levels of HDL cholesterol, and decreased the non-HDL cholesterol, by 50%. It has been speculated that short term treatment of humans with the same CETP inhibitor could result in a 40% to 45% increase in HDL cholesterol and a 15% to 20% decrease in LDL cholesterol.

Thus, it has also been suggested that inhibitors of CETP have the potential to inhibit atherogenesis by enhancing the rate of reverse cholesterol transport, the pathway by which cholesterol in peripheral tissues is transported to the liver for elimination in the bile. Thus, CETP offers a model target for raising HDL and inhibiting atherosclerosis. A compound JTT-705 (**29**) inhibits CETP activity by forming a disulphide



bond with protein. In cholesterol fed rabbits, (**29**) increased plasma HDL-C, decreased non–HDL-C and importantly caused a 70% decrease of aortic arch lesions [146]. A trifluoro-3-amino-2-propanol, (SC-795 **30)** is also reported to inhibit the CETP-mediated transfer of [3*H*]- cholesterylesters from HDL to LDL in human plasma (IC_{50} = 0.6μM) [147-149].

5.9. C-reactive Protein (CRP)

C- reactive protein is a powerful independent predictor of future cardiovascular risks [150] in any individual. This discovery reflects the pivotal role that proinflammatory processes play in atherogenesis and its complications [151]. CRP is an acute phase reactant largely produced by the liver in response to inflammatory cytokines such as interleukin-6 that have been viewed as inactive downstream markers of low grade vascular inflammation. Several other more sophisticated measures of cytokinase activation, cellular adhesion and immune and enzyme functions have also been found to predict future risks of cardiovascular events [152]. CRP predicts not only future incidents of myocardial infarction and cardiovascular death, but also the risks of ischemic stroke, sudden cardiac death, incident peripheral vascular disease and stenosis after percutaneous coronary intervention.

CRP is a member of the pentraxin family which consists of five noncovalently associated peptides surrounding, a central core binding site of bacterial and fungal polysaccharides. CRP acts as a member of the innate immune system activating the classical pathway of complement fixation and inducing phagocytosis. SAA (serum amyloid A) another acute phase protein is induced by the interleukins; IL-6 and IL-1 and tumor necrosis factor (TNFα) and is synthesized by the liver. SAA is an apoprotein, associated primarily with HDL, inducing matrix degrading enzymes and acting as a chemo attractant for monocytes, as well as, mediating lipid delivery to peripheral cells and removal of cholesterol from damaged tissues [153].

Moreover, in a recent head to head prospective comparison of CRP and LDL cholesterol among a group of 27,939 women it has been found that CRP is the stronger predictor of incident cardiovascular events and has added prognostic information at all levels of the Framingham risk score [154]. Perhaps even more importantly CRP and LDL cholesterol are minimally correlated and identify different individuals at risk for cardiovascular events. CRP has been found to localize within the complement membrane attack complex in early atherosclerotic tissue [155] and levels of mRNA encoding both CRP and certain complement factors are increased in atherosclerotic plaques [156], where smooth muscle cells and macrophages appear to be the main producers of CRP.

Although CRP concentrations as low as 5mg/l have been found to decrease nitric oxide production, the concentration of CRP shown to elicit many of these proinflammatory responses are typically just in excess of 5 μg/ml. The plasma concentrations of CRP are used to denote low, intermediate and high risk for primary prevention. Thus, concentrations of CRP used for clinical risk prediction appear generally lower than those shown to elicit proatherogenic responses. It is possible that circulating CRP levels do not truly reflect tissue concentrations and also locally concentrated CRP may be present in a sufficient amount to promote atherogenesis. Further research is required to address these issues.

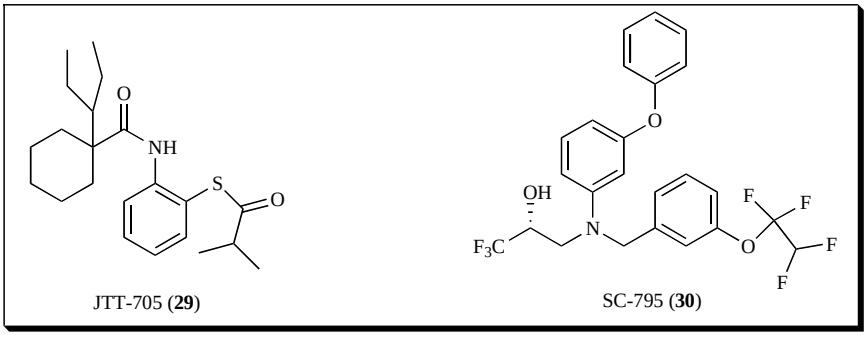
Thus, atherosclerosis is accelerated by both, by the direct effects of cytokines on endothelial cells and by indirect effects of cytokine on the liver, causing increased production of CRP and related factors [157]. CRP induces endothelial cell adhesion molecules and increases endothelial cell monocyte chemoattractant protein-I [158], inhibits nitric oxide synthesis [159], increases plasminogen activators (PA-I) expression [160] and leads to endothelial dysfunction [161] in atherosclerosis prone animals which overexpress CRP. This shows direct role of CRP in increased atherosclerosis. Similarly, there is evidence that SAA may be a mediator of atherosclerosis [162]. HDL particles containing SAA rather than apolipoprotenemia (apo)A show adherence to vascular proteoglycon *via* the tethering region of SAA, suggesting the existence of 'inflammatory HDL' [163].

5.10. Antioxidants

5.10.1. Lipid Oxidation in Atherogenesis [164]

Normally, receptor-mediated uptake of low-density lipoproteins (LDL), the major carriers of plasma cholesterol, is suppressed through down-regulation of LDLR (LDL receptor) expression in response to increasing cholesterol levels [165]. Therefore, an idea evolved that LDL may be subject to modification within the intima that converts it into a particle which is still internalized by macrophages. However, this modification may be *via* receptors, whose expression is not controlled by cholesterol loading. These receptors include the macrophage 'scavenger' receptors of class-A (SR-A) that bind LDL when its net surface charge is altered, chemically.

The average LDL particle contains one molecule of apolipoprotein B (apoB), 600 molecules of free cholesterol, 1600 molecules of cholesteryl ester, 700 molecules of phospholipids, 180 molecules of triacylglycerol and 10 molecules of α-



JTT-705 (**29**) SC-795 (**30**)

tocopherol (αTOH); the major endogenous antioxidant. During cell-mediated conversion of LDL into a high-uptake particle, all of these components are subject to oxidation. The 'oxidation theory' for atherosclerosis proposes that lipid and/or protein oxidation products are responsible for lesion formation/development.

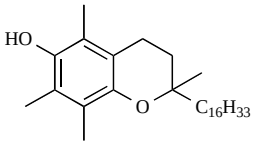
The major target for oxidation is suggested to be intimal low-density lipoproteins, due to the pro-atherogenic properties of *in vitro* oxidized lipoproteins. Lesion lipids contain products generated by both enzymatic and nonenzymatic oxidation reactions; the majority being generated nonenzymatically.

Besides, its potential role in foam-cell lipid accumulation, oxidized LDL has numerous other biological activities. Oxidized LDL stimulates the expression of VCAM-1 (vascular cell adhesion molecule-1) on endothelial cells, also it is directly chemotactic for monocytes, and it increases the expression of MCP-1 (monocyte chemotactic protein-1) by vascular cells and stimulates macrophage proliferation. These properties could promote the early inflammatory infiltration of monocytes into the vascular wall. In addition to its uncontrolled uptake, oxidized LDL also inhibits cholesterol export from macrophages due to direct effects of oxysterols on the cholesterol efflux machinery [166] and due to accumulation of undegradable cholesteryl esters in lysosomes. This can also contribute to foam-cell formation. Oxidation of LDL also increases its sensitivity to aggregation and to modification by sphingomyelinase, promoting further the likelihood of its intimal modification and uptake by macrophages. Oxidized LDL attenuates endothelium dependent relaxation of blood vessels, and can cause injury, apoptosis and necrosis of vascular cells [167]. This may lead to the release of lipids and lysosomal enzymes into the intima, promoting the progression of atherosclerotic lesions, such as the development of the acellular lipid core. In addition, oxidized LDL can promote macrophage and smooth muscle cell proliferation as well as, the expression and secretion of a variety of growth factors and cytokines from vascular smooth muscle cells, endothelial cells and macrophages. Components of oxidized LDL are also immunogenic and may additionally contribute to the chronic inflammatory components of lesion development.

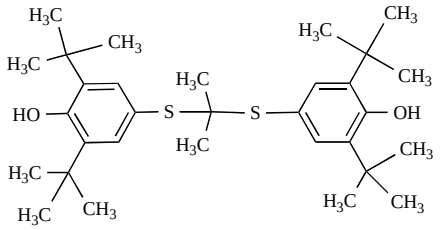
Both *in vitro* studies and epidemiology indicate that oxidized lipids may be involved in atherosclerosis. However, in humans *in vivo* lipid oxidation appears to progress in the presence of normal vitamin E levels, and antioxidant supplementation does not prevent atherosclerosis. This suggests that the oxidation of lipids in the vessel wall may be independent of vitamin E. However, two-electron oxidants are increasingly implicated in atherosclerosis, against which α-TOH is not effective. The increasing evidence of a role for HOCl (hypochlorite) in atherosclerosis raises the possibility that its primary target, protein oxidation is a more important contributor to lesion development than lipid oxidation. Further studies are required to identify unequivocally the oxidants involved in lesion oxidation and their primary targets, to allow better design of future antioxidant strategies [164].

5.10.2. Few Reported Antioxidants [168-173]

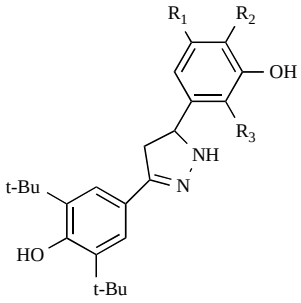
Many antioxidants have been developed to exhibit the antiatherogenic activities by inhibiting the foam cell formation in animal models [168]. Vitamin E (**31**) and probucol (**32**) as antioxidants have an untoward effect, of lowering serum HDL-cholesterol levels. Better results were seen by the introduction of a pyrazoline moiety in place of the disulphide linkage of probucol between its two phenyl rings. [169].



Vitamin E (**31**)



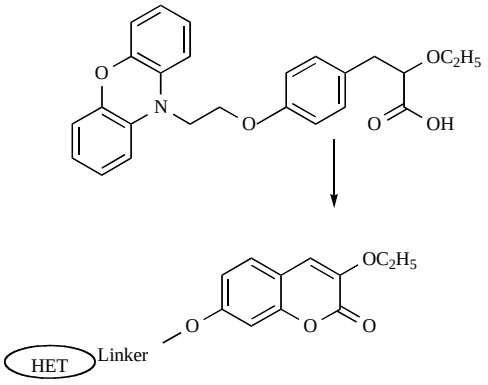
Probucol (**32**)



3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-5-(substituted-4-hydroxyphenyl)-2-pyrazolines (**33**)

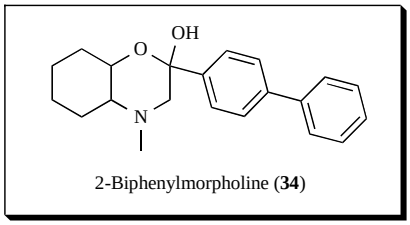
Thus, it has been demonstrated that 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-5-(substituted-4-hydroxyphenyl)-2-pyrazolines (**33**) are active inhibitors of LDL oxidation. These findings need further study to clarify the mechanism of antioxidant action of the pyrazolines in the oxidation of LDL [170].

Coumarins are known to have antioxidant potential quite similar to tocopherol (Vitamin-E). Lohray *et al.,* [171] have reported a few N-ethoxyphenyl derivatives of phenoxazine, (Scheme **1**) to exhibit good blood glucose lowering activity in experimental animal models. They have made changes in the pharmacophore by cyclizing the 2-ethoxypropanoic acid of the side chain to a coumarin moiety. Coumarin derivatives, having different heterocycles, attached to them with a linker of one or two carbon chain, show interesting triglyceride lowering activity (Scheme **1**) [172].



Scheme 1.

2-Biphenylmorpholine derivatives (**34**), structurally similar to some substituted morpholines inhibit the ferrous/ascorbate induced lipid peroxidation of microsomal membrane lipids with an IC_{50} value of 250μM, indicating themselves to be potentially antiatherogenic factors [173].



2-Biphenylmorpholine (**34**)

5.11. Diacylglycerol O-acyltransferase (DGAT)

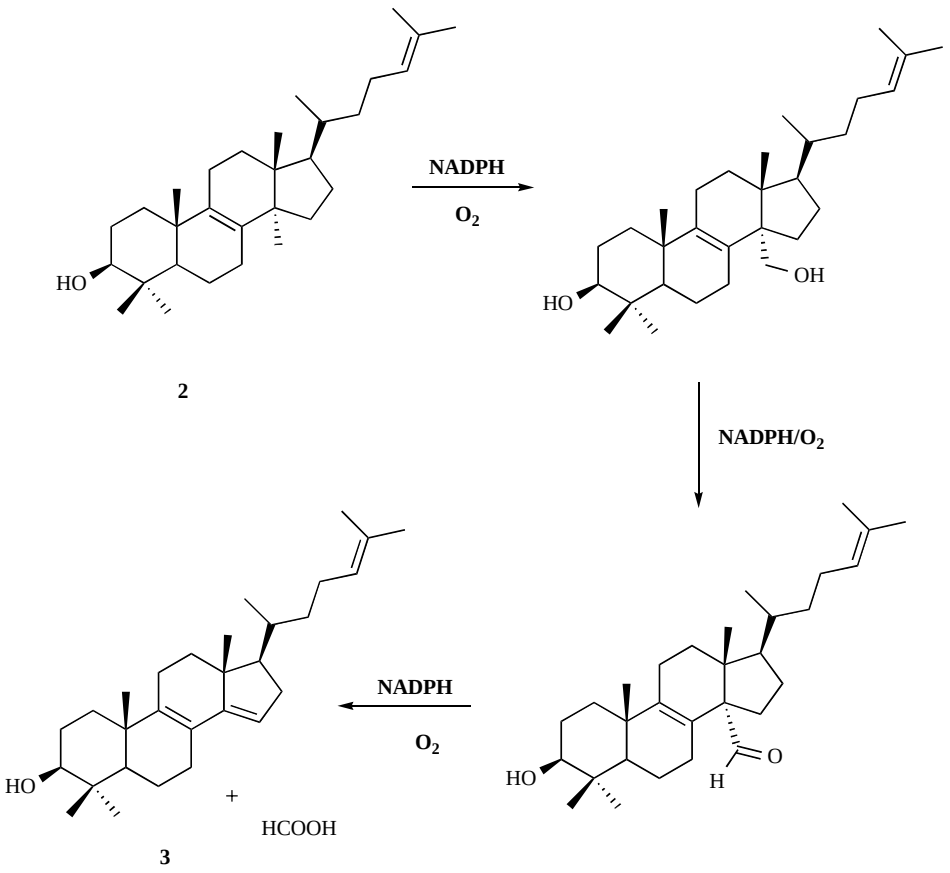
Considerable attention has recently been focused on the hypothesis that; defects in the intracellular triglyceride (TG) biosynthetic pathway may play a role in the pathogenesis of such pervasive conditions as obesity, NIDDM, and atherosclerosis. Several studies have documented the down regulation of lipoprotein lipase, hepatic triglyceride lipase, and VLDL receptors leading to depressed clearance and elevated plasma concentrations of triglyceride-rich lipoproteins. Although, much is known about the metabolic regulation of TG lipolysis, the regulatory mechanisms underlying the intracellular TG lipogenesis in adipocytes have yet to be fully elucidated [174-177]. The addition of fatty acid to diacylglycerol by acyl-CoA:1,2-diacylglycerol O-acyltransferase (DGAT) is the only step in TG synthesis unique to this biosynthetic pathway, and strong evidence exists for its regulation in rat liver and adipose tissue by phosphorylating-dephosphorylating activities present in a cytosolic fraction [178, 179] Although there is some controversy as to whether the rate-determining step for the *de novo* synthesis of TG is catalyzed by diacylglycerol acyltransferase (DGAT) or phosphatidate phosphohydrolase [180, 181], much of the convincing evidence favors DGAT [182, 183]. Using a specific inhibitor of DGAT in lysolecithin-permeabilized cells, Mayorek *et al.,* [182, 184] have demonstrated that the overall flux of substrate through the TG pathway is rate-limited by DGAT. DGAT is a microsomal enzyme located principally on the endoplasmic reticulum [185] and has been recently purified to near homogeneity by Andersson *et al.,* [186]. Two distinct forms of DGAT (DGAT-1 and 2) have thus far been identified. Haagsman *et al.,* [187] have shown that TG synthesis can be regulated independently of phospholipid synthesis by modulation of DGAT activity. These investigators have also presented strong evidence indicating that rat liver DGAT exists in two catalytic states, interconvertible by phosphorylating-dephosphorylating activities present in a cytosolic fraction. Thus, the investigation of the molecular mechanisms involved in the regulation of TG synthesis by pharmacological agents [188] and by novel effectors, such as Acylation Stimulating Protein (ASP) [189, 190], is of considerable interest. This protein markedly stimulates *de novo* TG synthesis in human skin fibroblasts and adipocytes [191, 192] by 1) stimulating the translocation of glucose transporters from an intracellular pool to the cell surface membrane [193], and by 2) increasing the flux of substrate through the triglyceride synthetic pathway, by increasing the V_{max} of the enzyme DGAT [194]. The stimulatory effect of ASP on the intrinsic activity (V_{max}) of the enzyme DGAT is mediated by a change in the phosphorylation state of this enzyme [189]. Although mice lacking DGAT are viable and can still synthesize triglycerides, their fat-pad weights are lower than wild-type control mice. In addition, DGAT-deficient mice are resistant to obesity when fed on a high-fat diet [195]. The recently cloned DGAT gene is a plausible candidate for the genetic study of obesity. Ludwig *et al.,* [196] identified a T79C single nucleotide polymorphism (SNP) in the DGAT gene associated with increased promoter activity.

5.12. Lanosterol 14α-Demethylase (14α-LDM)

Cholesterol is an essential component of cell membranes and a precursor for the steroid hormones and bile acids. It is also essential for the growth and function of yeasts and fungi. Two sources of cholesterol exist for the mammalian system: exogenous (*i.e*., dietary) sterols and endogenous sterols biosynthesized from acetyl-coA. Disorders of cholesterol biosynthesis and regulation can result in elevated serum cholesterol and atherosclerosis [197].

Lanosterol 14α-demethylase is the cytochrome P_{450} monooxygenase, which oxidatively removes the 14α-methyl groups of lanosterol (Scheme **2**) [198].

Inhibitors of cytochrome $P_{45014DM}$ are not only of interest as mechanistic probes of the enzyme, but also as potential



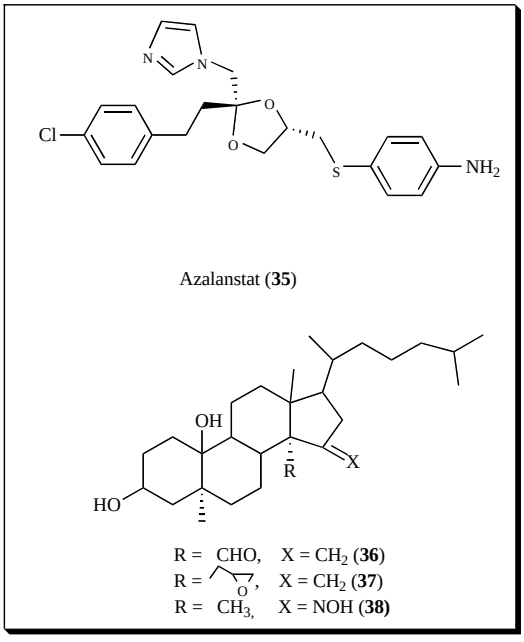
Scheme 2.

therapeutic agents for treatment of hypercholesterolemia and as antimycotics. A number of oxysterols that are suppressors of HMGR (HMGCoA Reductase) activity are catabolites of cholesterol [199]. However, cells which do not oxygenate cholesterol are still capable of regulating the activity of HMGR [200]. HMGR activity and sterol synthesis in these cells can be inhibited by known oxysterol suppressors of HMGR [201, 202]. These facts strongly suggest that one or more oxysterols, which are precursors to cholesterol, may be important in the regulation of cholesterol biosynthesis.

Oxysterols are generated in the biosynthesis of cholesterol during the oxidative removal of the three extra methyl groups (C-30, C-31, C-32) of lanosterol. Lanosterol analogs oxygenated at C-32 and their Δ^7 isomers have been shown to decrease the activity of HMGS (HMGCoA Synthase) in Chinese hamster lung cells [201].

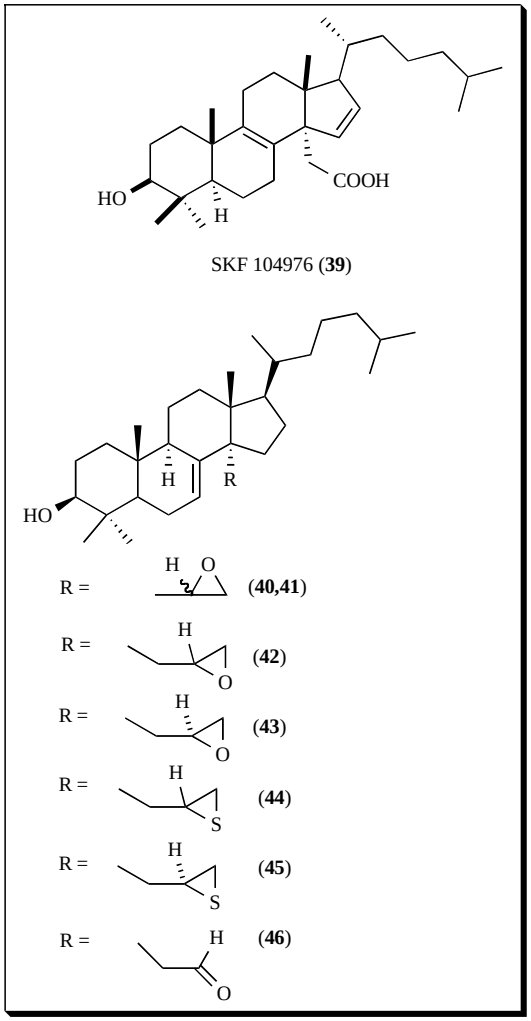
Azalanstat (RS-21607) (**35**), a synthetic imidazole, has been shown to inhibit cholesterol synthesis in HepG2 cells, human fibroblasts, hamster hepatocytes and hamster liver, by inhibiting the cytochrome P_{450} enzyme lanosterol 14 alpha-demethylase (LDM) [203].

Ethenyl lanosterol (**36)** is reported to function as an irreversible inhibitor of rat liver LDM [204], while the epoxide (**37)** is a competitive inhibitor (k_i = 0.6 μM) [205]. The oxime (**38)** is a modest inhibitor of LDM [(IC_{50}) = 55μM], but is an effective oral hypocholesteremic agent in hamsters [206].



A 32-carboxylic acid derivative of lanosterol, SKF 104976 (**39)** has been found to be a potent inhibitor of lanosterol 14 α-demethylase (14 α-LDM). The 14 α-LDM activity in a Hep G2 cell extract is inhibited to 50% by 2nM of a compound SKF 104976 (**39**). It is further suggested that upon inhibition of 14α-LDM by **39** a mevalonate-derived precursor which regulates HMGR activity, even when the

sterol synthetic rate is considerably reduced and when HMGR protein levels are very high [207].



The steroidal compounds, **40**-**46**, are shown to inhibit the biosynthesis of sterols downstream from lanosterol. A range of inhibitory potencies has been observed, and the key enzyme being inhibited is believed to be cytochrome $P_{45014DM}$. Inhibitor efficacy was readily correlated with non-metabolized [24,25-3*H*]dihydrolanosterol, formation of 4,4-dimethyl-cholest-8(9)-en-3ß-o1 and formation of lathosterol, a sterol believed to be an excellent indicator of whole body cholesterol biosynthesis in humans [208].

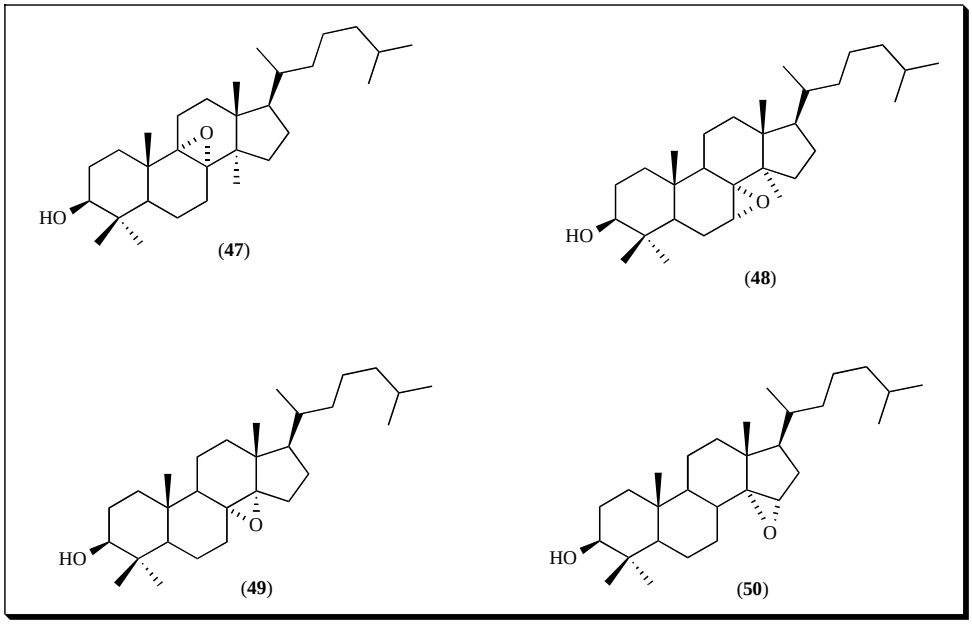
Examination of the metabolic profiles for cholesterol biosynthesis in the presence of the inhibitor series (**40-46)** suggests that these compounds indeed exert their inhibitory effects, in a dose-dependent manner, on cytochrome $P_{45014DM}$. Furthermore, these inhibitors have a marked effect on cholesterol biosynthesis, making cytochrome $P_{45014DM}$ a suitable candidate for therapeutic control of cholesterol biosynthesis *in vivo.*

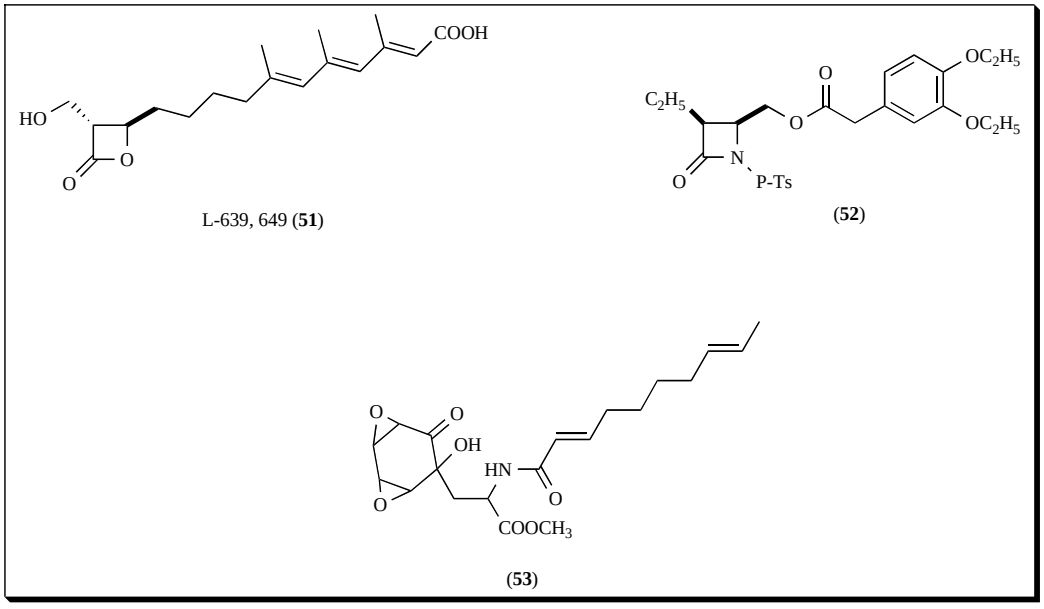
The inhibitors in series **47-50** are less potent blockers of both cytochrome $P_{45014DM}$ and downstream cholesterol biosynthesis. Unlike compounds, **40-46** they lack a nuclear double bond, which may reduce their binding affinity for cytochrome $P_{45014DM}$ and hence their potency as inhibitors. Fischer *et al.,* [209] have concluded that, since unsaturated and saturated dihydrolanosterols are both competitive inhibitors of cytochrome $P_{45014DM,}$ it is unlikely that the nuclear double bond is actually involved in the binding of the substrate to the active site of cytochrome $P_{45014DM}$. However, they have suggested that the position of the nuclear olefinic group does affect both, the orientation of the l4α-methyl group of these lanosterols and their reactivity towards the 14α-demethylation process.

Thus, inhibitors of 14α-LDM can be good therapeutic agents for antihyperlipidemia treatment.

5.13. HMG Synthase

HMG synthase catalyses the cholesterol biosynthetic step just prior to the reduction of HMG-CoA and thus its inhibi-





tion is also one of the strategies for antihyperlipidemic therapy. Reports have shown L-639, 649 (**51**) to be a potential HMG synthase inhibitor [210]. Its tosyl lactam (**52**) with an IC_{50} value of 2.0 nM is also identified as potent inhibitor of HMG synthase [211].

A synthetic β-lactone, trans-DU-6622 (**53**), a mixture of (2R, 3R) and (2S, 3S)- β-lactones, was found to inhibit HMG- synthase (IC_{50}: 0. 15 μM) and pancreatic lipase (IC_{50}: 120 μM). The effects of the optically pure isomers of **53** on the two enzymes have been compared. The (2R, 3R)-isomer has been shown to be a highly specific inhibitor of HMG-synthase (IC_{50}: 0.098 μM *vs* 270 μM for pancreatic lipase), while the (2S, 3S)-isomer markedly increases the specificity of lipase inhibition (IC_{50} : 27 μM *vs* 31 μM for HMG- synthase). The (2R, 3R)-β-lactone is responsible for the specific inhibition of HMG- synthase, while the (2S, 3S)-β-lactone is responsible for the inhibition of pancreatic lipase [212].

5.14. Squalene Synthase (SS)

Squalene synthase catalyses the reductive dimerization of two molecules of farnesyl pyrophosphate to form squalene. Further, these inhibitors also exhibit triglyceride lowering activity comparable or better than fibrates. Inhibition of this enzyme has also been demonstrated to upregulate LDL receptor activity, thus inhibition of SS can be a good therapeutic strategy for lipid lowering.

A potential inhibitor of SS is BMS-188,494 (**54**) a prodrug, having the ability to lower cholesterol in rats after oral administration, a model insensitive to lipid lowering with statins [213, 214].

Thus, it appears that inhibition of this enzyme may also decrease circulating LDL levels by inducing LDL receptors. There may, however be important differences between the other effects of squalene synthase inhibitors and those of HMG CoA reductase, because squalene synthase which acts down stream of mevalonate, is the first committed step of hepatic cholesterol biosynthesis at the final branch point of the cholesterol biosynthetic pathway to avoid the effects associated with decreased formation of isoprenoids and other intermediates and metabolites in the pathway beyond HMG-CoA reductase.

Compound, RPR 107393 (**55**) and its R and S enantiomers are potent inhibitors of rat liver microsomal squalene synthase, with IC_{50} values of 0.6 to 0.9 nM. One hour after oral administration to rats, RPR 107393 inhibited *de novo,* ^{14}C cholesterol biosynthesis from ^{14}C mevalonate in the liver with an ED_{50} value of 5 mg/kg. The R and S enantiomers of RPR 107393 (20 mg/kg *p.o., q.d.* for 7 days) reduced plasma LDL cholesterol and TG by 50% and 43%, respectively, whereas HDL cholesterol was unchanged. It is an orally effective hypocholesterolemic agent in rats and marmosets & has greater efficacy than lovastatin or pravastatin [215].

A prodrug compound, ER-28448 (**56**), a potent and selective inhibitor of squalene synthase, inhibits it in rat liver microsomes with an IC_{50} value of 3.6 nM. Another prodrug ER-27856 (**57**) although less active than ER-28448 (**56**), more potently inhibits cholesterol biosynthesis in rat hepatocytes and also orally inhibits *de novo* cholesterol biosynthesis in Sprague-Dawley rats, with an ED_{50} value of 1.6 mg/kg [216, 217].

Another squalene synthase inhibitor YM-53601 (**58**) lowers not only plasma cholesterol, but also plasma triglyceride levels. It equally inhibits squalene synthase function in hepatic microsomes prepared from several animal species and suppresses cholesterol biosynthesis in rats (ED_{50} 32 mg/kg). In rhesus monkeys, when dosed at 50 mg/kg, twice daily for 21 days, it decreases plasma non-HDL-C by 37% [218].

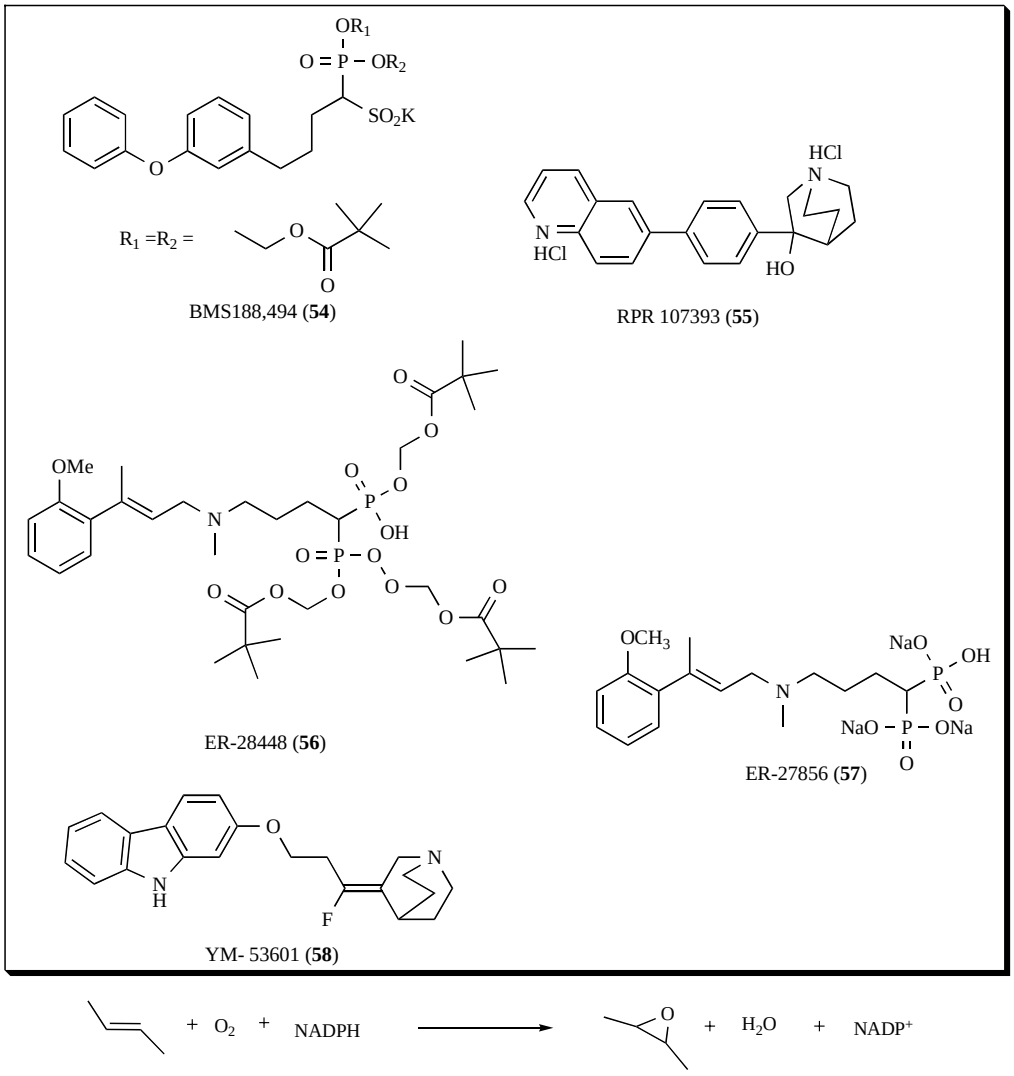
5.15. Squalene Epoxidase (SE)

Therapeutic success of HMGR inhibitor statins has distinctly established inhibition of *de novo* hepatic cholesterol synthesis as an effective approach to lower plasma LDL-

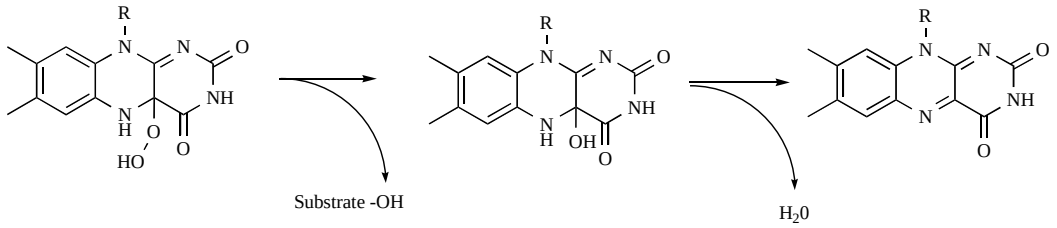
cholesterol, the major risk factor for atherosclerosis and coronary heart disease. Howevers statins also inhibit the synthesis of several non-sterols *e.g*., dolichols and ubiquinone, leading to the side effects observed with statins. This has prompted researchers to target selective cholesterol synthesis beyond farnesyl pyrophosphate. The enzymes squalene synthase, squalene epoxidase and oxidosqualene cyclase have been identified as potential targets. Of these squalene epoxidase catalyses the conversion of squalene to 2,3-oxidosqualene. Although it has been extensively exploited for antifungal drug development, it has received little attention as a target for hypocholesterolemic drug design. This enzyme is an attractive step for pharmacotherapeutic intervention as it is the secondary rate limiting enzyme and blocking cholesterol synthesis at this step may result in accumulation of only squalene which is known to be stable and non toxic. This enzyme catalyses another rate limiting step in the biosynthesis of cholesterol, *viz*., squalene epoxide from squalene. Squalene monooxygenase, earlier known as squalene epoxidase catalyzes the insertion of an oxygen atom across a carbon-carbon double bond to form an epoxide (Scheme **3**) [219].

Flavoprotein monooxygenases accomplish this oxygenation by forming a flavin hydroperoxide at the enzyme active site, which then transfers the terminal oxygen atom of the hydroperoxide to the substrate. The remaining "hydroxyflavin" then reoxidizes with the release of water (Scheme **4**).

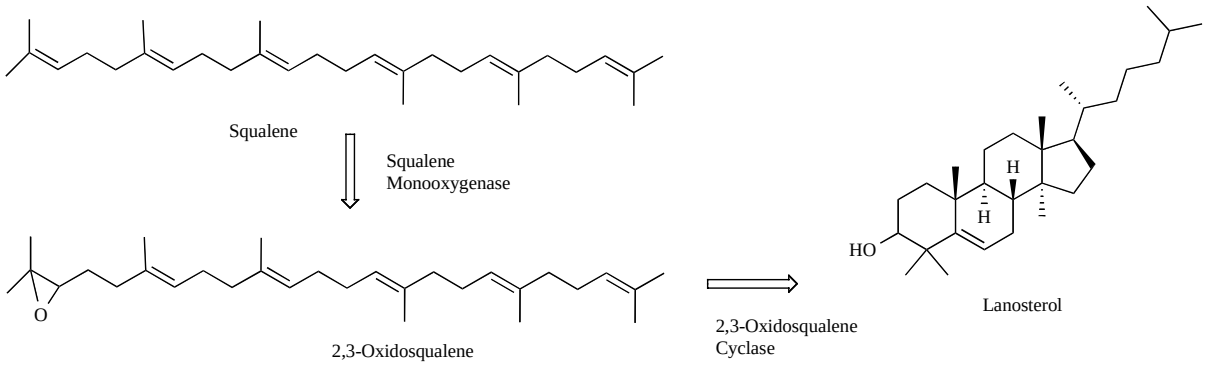
Squalene monooxygenase presumably utilizes this same mechanism, but differs from other known flavin monooxygenases in that the oxygen is inserted as an epoxide rather than as a hydroxyl group. Indeed, this type of reaction is more typical of heme monooxygenases (the cytochrome P_{450}) and some pterin-dependent hydroxylases. Squalene monooxygenase contains a loosely bound FAD flavin and obtains electrons from NADPH-cytochrome P_{450} reductase, rather than binding the nicotinamide cofactor NADPH, directly. This also distinguishes squalene monooxygenase from other flavin monooxygenases. Because of the difficulty in purifying this microsomal enzyme, and its low abundance, even in liver, little is known about this enzyme. Its low abundance and low specific activity suggest that squalene monooxygenase may be the rate-limiting component in cholesterol biosynthesis [220].



Scheme 3.



Scheme 4.



Scheme 5.

Squalene monooxygenase is regulated at the transcriptional levels in response to sterol levels in the cell. Although HMG-CoA to mevalonic acid conversion by HMGCoA reductase is traditionally considered to be the regulating step in cholesterol synthesis, it is now clear that squalene synthase and squalene monooxygenase are also important regulatory points. The conversion of squalene, a 30-carbon linear isoprenoid, to lanosterol, a tetracyclic compound (Scheme **5**), occurs in two steps as first elucidated by Corey and van Tamelen, in the late 1960's [221,222]. Yamamoto and Bloch [223] showed that the first step, catalyzed by squalene monooxygenase, required both the microsomal and cytosolic fractions of liver, along with NADPH and O_2.

Bloch and Ono [224] have shown that squalene monooxygenase is bound to the endoplasmic reticulum of cells in association with NADPH-cytochrome P_{450} reductase, its electron transfer partner. The cytosolic fraction has been shown to be composed of phospholipids and a 45 kDa protein termed 'supernatant protein factor' or 'sterol carrier protein' [225-227]. Supernatant protein factor (SPF) was recently cloned and shown to be a member of the cytosolic lipid binding/transfer protein family, including yeast phosphatidylinositol transfer protein [228]. The exact role of SPF remains unclear; however it can be replaced by the nonionic detergent Triton X100 for *in vitro* assays.

Activity studies indicate that squalene epoxidase is expressed at very low or negligible levels in most non-cholesterolgenic tissues, and is found in greatest abundance in the liver, followed by the gut, skin, and neural tissues [229].

Squalene monooxygenase, like HMG-CoA reductase, exhibits diurnal variation in activity, with activity highest during the night. Recent studies have shown that enzyme activity is regulated by changes in gene transcription in response to sterol levels, including the oxysterol; 25-Hydroxycholesterol, also down-regulates HMG-CoA reductase and LDL receptor expression, which could be a physiological feedback regulator of the cholesterol biosynthesis pathway [230]. Put in perspective squalene epoxidase appears to be undervalued target which merits attention for development of better hypocholesterolemic drugs [231].

The most effective inhibitor of mammalian squalene monooxygenases to date is NB-598 (**59**), developed at Banyu Pharmaceutical Co., Japan This fungal-derived natural compound is a competitive inhibitor of squalene monooxygenase in human HepG2 cells with a k_i of 0.68 nM. It effectively reduces serum cholesterol in dogs with no apparent adverse effects [232, 233]. No studies have yet been reported in humans.

The 1,1-difluorosqualene, (**60**), is orally active in mice as indicated by dose dependent reductions in hepatic cholesterol synthesis [234]. Equivalent *in vitro* potency is seen with the cyclopropylamino derivative (**61**), against rat hepatic SE [235].

A variety of chemical compounds found in edible and medicinal plants have recently been shown to be potent and selective inhibitors of squalene monooxygenase. Recently, cholesterol-lowering effect of green tea has been attributed to potent squalene epoxidase inhibition. These facts strengthen optimism for developing clinically safe squalene epoxidase inhibitors. It has been reported that green tea polyphenols are particularly potent inhibitors of the recombinant rat enzyme [236]. The presence of a galloyl group (3,4,5-trihydroxybenzoyl) is shown to be necessary for inhibition. Epigallocatechin-3-O-gallate (EGCG) (**62**), the major green tea polyphenol, has a k_i of 0.74 μM. The major metabolites of EGCG are also inhibitory. Although a typical

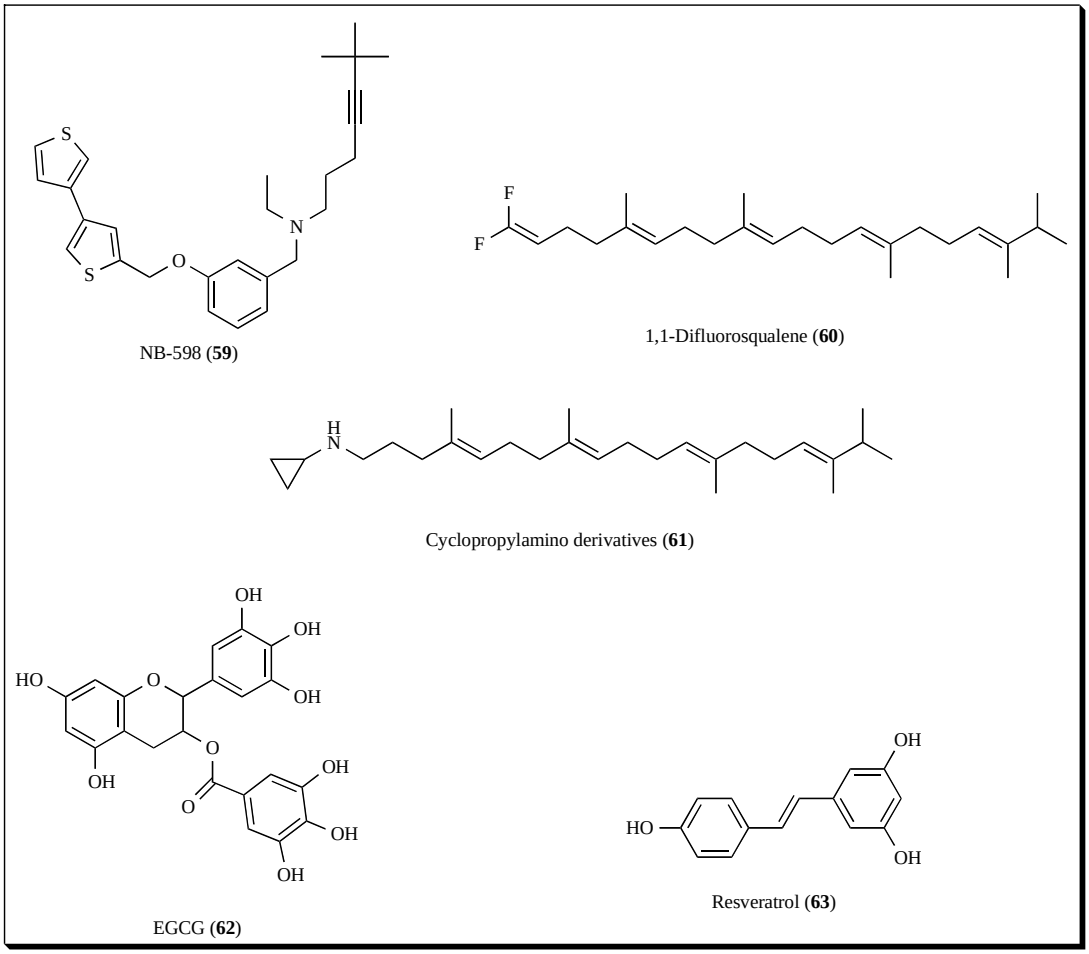
cup of green tea contains 100 mg of EGCG, the low bioavailability and 2-3 hr half-life suggests that significant tea consumption would be necessary to obtain therapeutic levels [237]. EGCG is a noncompetitive inhibitor of squalene monooxygenase, and may act by scavenging the reactive oxygen species formed at the active site of the enzyme (the flavin 4a hydroperoxide). Other plant extracts that contain galloyl esters, including *rhubarb* and the Chinese herb *fo-ti* (*Polygonum multiflorum*), have also been found to inhibit squalene monooxygenase and reduce serum cholesterol [236]. Additional studies have identified several synthetic galloyl esters as potent inhibitors of squalene monooxygenase, including dodecyl gallate, with a k_i of 33 nM [238]. Dodecyl gallate, and other synthetic alkylgalloyl esters, are widely used as antioxidant food additives.

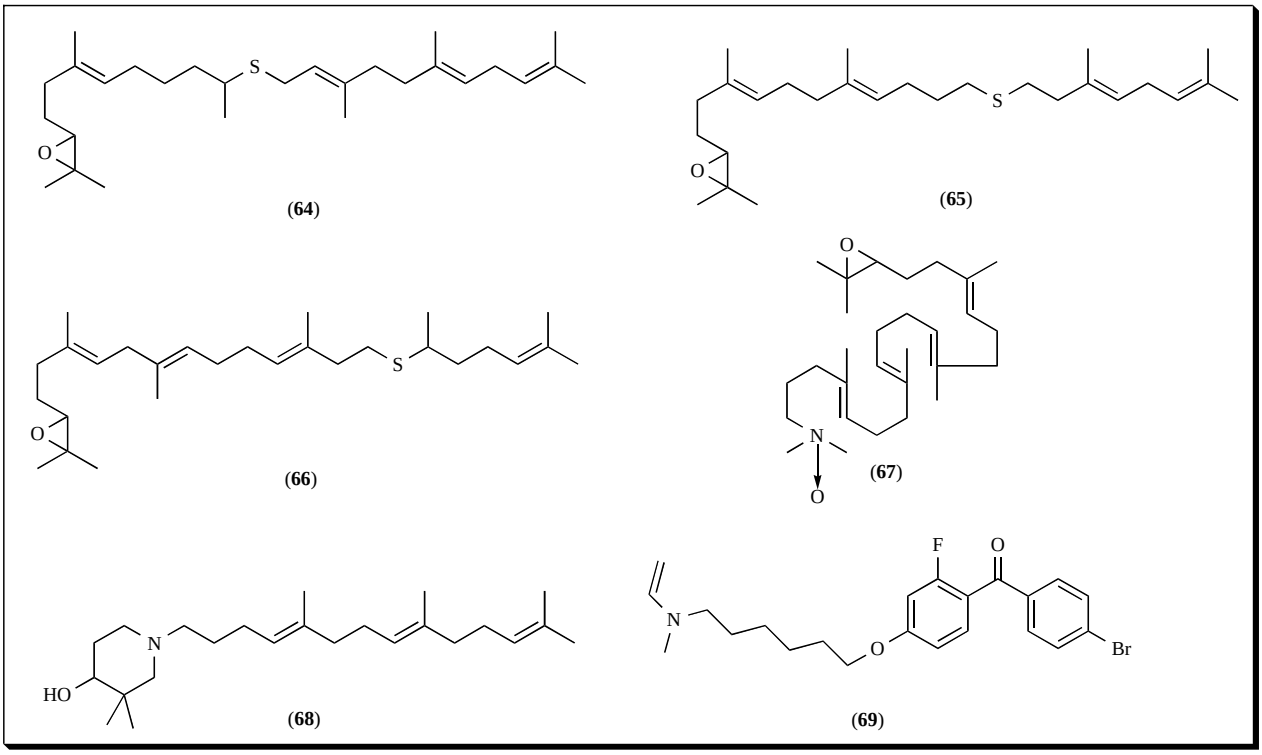
Resveratrol (trans-3,4',5-trihydroxystilbene) (**63**), a polyphenol found in grape skins and red wine, has also been reported to lower cholesterol and prevent cardiovascular disease [239]. It has been found that resveratrol is a modest inhibitor of squalene monooxygenase, with a k_i of 35 μM with respect to squalene [240]. As with the galloyl esters, resveratrol is a reversible, noncompetitive inhibitor of the enzyme.

Garlic is also reputed to lower blood cholesterol and have a variety of beneficial cardiovascular effects; 0.5% (final concentration) aqueous extract of fresh garlic inhibits greater than 90% of recombinant human squalene monooxygenase activity *in vitro* [241]. Because inhibition by garlic is irreversible, it is likely to act by a different mechanism than the polyphenols. One or more of the many oxidized sulfur compounds in garlic are likely to bind to SE and permanently inactivate it. S-allylcysteine is abundant and principal component of garlic and is one of the potent inhibitors of SE.

5.16. 2,3-Oxidosqualene Lanosterol Cyclase

Cyclization of 2,3-oxidosqualene to lansterol mediated by 2,3-oxidosqualene-lanosterol cyclase (OSC) [242] involves the formation of the protosterol cation and its backbone rearrangement to lanosterol which proceeds *via* distinct carbocationic intermediates. It has been proposed that a stereo controlled delivery of “point charge" nucleophiles by this enzyme can stabilize each cationic intermediates. The cyclization process has been the subject of numerous biomimetic studies. For several decades OSC have been the target of inhibition studies. Inhibition of OSC has been achieved by substrate analogues, as well as, mimics of the presumed carbocationic intermediates. Recently, attention has been focused on the design of mechanism based inactivators. Squalenoid epoxidase vinyl ethers have been examined as possible suicide inhibitors. The cyclization of such substrate should form C-20 carbocations, which could be stabilized through the formation of an oxocarbenium ion. The latter could interact with an active site nucleophile producing irre-





versible enzyme inhibition. In fact the vinyl ethers containing squalene oxides are competitive inhibitors, *i.e.,* 22, 23-dihydro-20-oxa-2,3-oxidosqualene has shown a k_i of 60 μ M and IC_{50} of 80 μ M for rat liver OSC.

As an overall view, inhibition of this non-rate limiting step is unlikely to result in effective inhibition of cholesterol biosynthesis. Indeed, upregulation of LDL receptors has not been reported with these compounds and they may have this potential only in combination with other hypocholesterolemic agents. 2,3-Oxidosqualene cyclase has been targeted with the rationale that partial inhibition of this enzyme will result in the accumulation of 2,3-oxidosqualene and 2,3:22,23-squalene dioxide which is further metabolized to 24, 25-epoxycholesterol a potent repressor of HMG-CoA reductase leading to hypocholesterolemic effect without accumulation of oxysterols. A series of novel sulfur-substituted oxidosqualene (OS) analogues (**64-66**), have been synthesized and evaluated as OSC inhibitors. In these analogues, C-11, C-15, or C-18 in the OS skeleton have been replaced by sulfur. The sulfur position in the OS skeleton have been chosen to disrupt one or more key processes involved in cyclization, like (a) the folding of the B-ring into a boat conformation, (b) the anti-Markovnikov cyclization leading to the C-ring, or (c) the formation of the D-ring during the lanosterol biosynthesis. These compounds are potent inhibitors of mammalian OSC's (IC_{50} = 0.05-2.3 μM for pig and rat liver OSC) [243]. The S-18 analogue has shown to exhibit most potent inhibition of the rat liver enzyme (IC_{50} = 50 nM) and also shown potent, selective inhibition of the fungal enzyme (IC_{50} = 0.22 nM). Its k_i values ranges from 0.5 to 4.5 μM for pig OSC.

Two new azasqualenoid derivatives bearing a 22, 23-epoxy-2-aza-2,3-dihydrosqualene and of its N-oxide derivative (**67**), have been studied using rat and pig liver microsomal preparations, using a solubilized, partially purified squalene 2,3-oxide cyclase. All the compounds exhibit a non-competitive type of inhibition. Strong inhibition is obtained with an amide such as azadecalin IC_{50} = 0.7μM [244]. Roughly equivalent activity is observed with the monocyclic analog (**68)** [245], another new OSC inhibitors, Ro 48-8.071 (**69**), has shown effective lowering of plasma cholesterol in hamsters and squirrel monkeys, when compared to that by simvastatin [246].

5.17. Farnesoid X Receptor

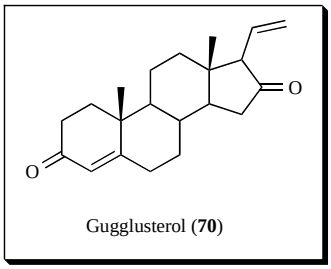
Nuclear receptors (NR's) are ligand-dependent transcription factors that control many biological functions, such as cell growth, differentiation, embryonic development, and metabolism. Upon activation by binding of small lipophilic molecules, such as steroids and thyroid hormones, retinoids, vitamin D, as well as dietary and endogenous lipids, NRs interact with coactivators to modulate directly the expression of responsive genes involved in development, reproduction and metabolism [247-249]. Todate, though 48 human nuclear receptors have been identified, about one third of them have no characterized ligands [250,251]. Recently, attention has been focused on the NR1H and NR1I subfamilies of the nuclear receptors, because of the increasing incidence of metabolic syndrome and the role of these receptors in the control of cholesterol and bile acid metabolism.

These receptors include the Farnesoid X receptor (FXR). It is activated by cholesterol metabolism end-products, bile acid derivatives, (such as primary bile acids and secondary bile acids, bile alcohols) and synthetic ligands. Primary bile acids include, chenodeoxycholic acid (CDCA) and cholic acid (CA), while secondary bile acids include deoxycholic acid (DC) and lithocholic acid (LCA). FXR is mainly ex-

pressed in the liver, intestine and kidney [252-255] and it plays an essential role in bile acid/cholesterol homeostasis [256,257]. FXR belongs to the steroid hormone receptor superfamily. However, crystal structure studies have suggested that bile acids bind FXR with their steroid backbone flipped head to tail in the reverse orientation [258, 259] which is different in manner compared to the binding of other steroid hormones to their receptors [260, 261]. Bile alcohols are produced as intermediates in the bile acid synthetic pathway in mammals and as end-products of cholesterol catabolism in most evolutionarily primitive vertebrates [262]. The bile alcohol, 5 α-cyprinol was originally isolated from the bile of *Cyprinus carpi* [263], the asiatic carp, and 5a-bufol was isolated from the bile of lungfish [264, 265] and frogs [266]. FXR activation has been shown to repress the expression of cholesterol 7 α-hydroxylase (CYP7A1), a rate-limiting enzyme in the bile acid biosynthetic pathway, by inducing an orphan nuclear receptor, the small heterodimer partner (SHP) [267]. FXR is a very different nuclear receptor from liver X receptor (LXR), with a different role in lipid metabolism. Fundamentally, FXR is a bile acid sensor that functions to protect cells and organs, especially the liver and the intestine, against bile acid toxicity [268]. Bile acids are the major ligands for FXR, and activating of FXR have a variety of transcriptional effects, many of which are geared towards preventing, uptake as well as promoting excretion of bile acids. One effect of FXR activation is the down regulation of Cyp7A1 and thus bile acid synthesis; which is accomplished through induction of SHP (short heterodimer partner) which then represses Cyp7A1 transcription. A second effect of FXR activation is downregulation of the hepatic bile acid import pumps NTCP and OATP (Na-dependent taurocholate cotransporting polypeptide and organic anion transporting polypeptide), thus reducing the import of bile acids from the plasma compartment into the hepatocyte. A third effect of FXR activation is upregulation of the hepatic bile acid export pumps BSEP (bile salt export protein) and MRP2 (multidrug resistance-associated protein–2), thus increasing the export of bile acids out of the hepatocyte into the bile. A fourth effect of FXR activation is the induction of pathways responsible for detoxification of bile acids. FXR activation by bile acids apparently has evolved out as a defense mechanism to protect liver and the intestine against the toxicity of bile acids. The role of FXR in LDL metabolism is a bit more complex. Bile acid sequestrants (BAS) have long been used as LDL-lowering drugs, and are known to cause increased triglyceride levels. BAS prevent intestinal reabsorption of bile acids, thus reducing the endogenous ligands for FXR and therefore functionally acting to reduce FXR activation. The fact that BAS have the effect of lowering LDL cholesterol suggests that a synthetic FXR antagonist might reduce LDL cholesterol levels.

Thus, therapeutically targeting FXR to improve dyslipidemia is complex. FXR agonists are likely to be effective triglyceride-lowering drugs and they may also have potential benefits in reducing elevated glucose. FXR antagonists or modulators may have a role as LDL-lowering drugs. FXR modulators having antagonist properties may be a potential of increasing apoA-I expression. Clinical studies of natural and synthetic FXR agonists and modulators will be necessary in future to address these issues.

Gugglusterol (**70**), which is available as dietary supplement in the US, is shown to reduce plasma LDL cholesterol by about 15–18% and triglycerides by about 25–30% [269].



Gugglusterol (**70**)

5.18. Sterol Regulatory Element Binding Proteins (SREBPs)-Cleavage Activating Proteins

Sterol regulatory element binding proteins (SREBPs) are a family of transcription factors that regulate cellular cholesterol and fatty acid homeostasis by responding to the cellular levels of free cholesterol [270, 271]. There are three SREBP isoforms designated as SREBP-1a, -1c and -2. SREBP-1a and 1c are encoded on the same gene located on human chromosome 17. While SREBP-2 is derived from a second gene located on human chromosome 22. SREBPs bind as dimmers to sterol regulatory elements (SREs) in the promotion of a large number of genes mostly activating their transcription. Sequences binding SREBP appear to be rather heterogenous, although there is some overlap in the genes regulated by SREBPs, SREBP-1c is thought to be the main of the regulator of genes involved in fatty acid synthesis, where as SREBP-2 regulates the gene involved in cholesterol biosynthesis. SREBP-1a is belived to activate genes involved in both processes [272].

Hepatocytes have LDL receptor gene that contains SRE, which can be activated by nuclear translocation of an active form of sterol regulatory binding proteins (SREBP-1 & SREBP-2) from the Golgi apparatus. The transport of SREBP to Golgi apparatus from sarcoplasmic reticulum (where it is normally located) requires the activity of a chaperon protein called sterol-regulatory element binding protein cleavage activating protein (SCAP). When SCAP senses a decrease in cellular cholesterol, it gets activated to transport SREBP to the Golgi apparatus for activation and from there the active SREBP moves into the nucleus to stimulate LDL receptor gene transcription, leading to the enhanced expression of LDL receptor, which leads to decrease in serum LDL cholestrol levels. Glaxo-Smith Kline has developed compounds which act as direct activating ligands for SCAP, leading to over expression of LDL receptors, thereby reducing levels of LDL, VLDL & cholesterol in the blood [273, 274].

5.19. Synthetic Apo E Related Peptides

Human plasma apo E is a 299-amino-acid protein (Mw. 34200), constituted by a single polypeptide chain. In humans, apo E is mainly produced by the liver and is secreted as an O-glycosylated protein [275]. It is associated with cholesterol-rich lipoproteins and mediates their uptake by the liver and peripheral tissues [276] ApoE is essential for the metabolism of VLDL, IDL, as well as, chylomicron and

VLDL remnants. A direct inverse relationship between circulating levels of plasma apoE and cholesterol has been documented. In humans, mutations of apoE or its complete deficiency results in greatly increased susceptibility to the development of atherosclerosis [277].

Apo E is present as three major isoforms, named apo E_2, E_3, and E_4 [278, 279]. Apo E_3 is the most frequent isoform in the general population. The molecular basis of apo E polymorphism is cysteine-arginine interchanges. Apo E_3 contains a single cysteine at the residue 112 and an arginine at position 158; apo E_2 contains cysteine residues at both positions 112 and 158; and apo E_4 contains arginine residues at both positions. This polymorphism leads to the presence of six different phenotypes in the human population: three homozygous (E3/3, E2/2, and E4/4) and three heterozygous (E2/3, E2/4, and E3/4). Although, apoE is one of the best-characterized apolipoproteins in terms of structure-function relationships, the particular structural properties of each isoform that explain the differences in reactivity still remain poorly understood.

ApoE binds to the LDL receptor, which has been shown to be essential for high affinity hepatic clearance of plasma cholesterol. At least two other cellular receptors bind apoE, a receptor expressed by the liver that binds to the apoE enriched remnant particles, currently thought to be the LRP/a2-macroglobulin receptor, and a VLDL receptor expressed predominately by the heart that binds to the apoE containing triglyceride-rich lipoproteins [280, 281]. Additional apoE receptors have been functionally defined and may or may not be related to the cloned receptors, LDL, LRP/cr2-macroglobulin and VLDL receptors [282].

In addition to its role in the transport of cholesterol and the metabolism of lipoprotein particles, apo E has several other functions in humans [283], *e.g*., immunoregulation [284], nerve regeneration [285], and the activation of several lipolytic enzymes (hepatic lipase, lipoprotein lipase, and lecithin:cholesterol acyltransferase) [286, 287]. Of particular interest, is apo E which serves as a ligand for several cell receptors. As is well accepted, specific interaction between apo E and the LDL-receptor is an essential mechanism controlling the removal of apo E-rich lipoproteins (VLDL, chylomicron remnants, and IDL), and thus determines the homeostasis of cholesterol and triglycerides [288]. Recent studies have shown that the LDL-receptor, which is expressed in several tissues, including the liver, plays a major role in the uptake of VLDL and remnants from plasma *in vivo* [289]. Genetic polymorphism of apo E explains 14-17% of the genetic variability of plasma cholesterol concentrations [290, 291]. Interestingly, the three common apo E isoforms have different affinities for the LDL-receptor. Apo E_3 and E_4 have the same affinity for this receptor, whereas apo E_2 shows defective binding activity, corresponding to 1% of that of the two other isoforms. VLDL and remnants containing apo E_2 are slowly removed from the plasma and induce an up-regulation of the liver LDL-receptor and thus a low concentration of plasma cholesterol. VLDL-apo E_4 particles are removed faster from plasma than VLDL-apo E_3 particles, inducing a down-regulation of the LDL-receptor. VLDL-apo E4 phenotype is thus associated with a higher concentration of circulating cholesterol. More recent studies have shown that apo E-rich lipoproteins act as ligands for receptors other than the LDL-receptor [292]. The LDL-related receptor protein (LRP) is expressed in several tissues, including the liver and the brain. Herz [280] has suggested its major role to be in the removal of j3-VLDL and intestinal chylomicrons remnants. However, this receptor also has a panel of very different ligands, such as a2-macroglobulin, and its exact contribution to the uptake of apo E-rich lipoproteins *in vivo* is difficult to assess [289, 293]. *In vitro* experiments with fibroblasts lacking the LDL-receptor have demonstrated the presence of another receptor, which is activated by oleate [294]. This complex, organized as two subunits (115 and 85 kDa) shows high affinity for both chylomicrons and VLDL and for apo E-enriched liposomes. This receptor, named as lipolysis-stimulated receptor, does not bind LRP ligands and shows only low affinity for LDL-apo B and VLDL-apoE_2. The lipolysis-stimulated receptor is localized mainly in the hepatocyte plasma membrane; its physiological role in the uptake of triglyceride-rich lipoproteins seems to be directly related to lipoprotein lipase activity [294]. Apo E-VLDL has been shown to interact with a fourth receptor, called the VLDL-receptor. The human VLDL-receptor shares 97% homology with the rabbit VLDL-receptor and 76% homology with the human LDL-receptor [295]. Its physiological role remains poorly understood. However, studies of the tissue distribution of the corresponding mRNA have demonstrated a high expression in tissues with significant fatty acid metabolism, such as muscle cells, adipocytes, heart, brain, and placenta. One important field of investigation in lipoprotein metabolism can be the determination of the respective contribution of each apo E receptor to the clearance of the apo E-containing lipoproteins and the effect of apo E polymorphism on these phenomena *in vivo*. The structure of the apo E isoforms may explain the different affinities of this apolipoproteins for their different receptors in both physiological and pathological conditions

Datta *et al.,* [296] have synthesized a dual-domain peptide, AchE18A-NH2, in which the arginine rich heparin-binding domain of apolipoprotein E (apo E) (residues141-150) is covalently linked to an 18 amino acid class A amphipathic helix with a high lipid affinity. This peptide, when administered intravenously to apo E knockout mice, reduced plasma cholesterol levels by 88% at 6 hours and by 30% at 24 hours. This peptide associates with LDL and VLDL and results in their rapid uptake by liver cells *via* a heparin sulfate proteoglycan facilitated pathway, opening up a novel approach to treating hypercholesterolemia. Further investigations into this peptide are needed to determine its safety and efficacy.

CONCLUSION

The annual global morbidity and mortality figures attributed to the risk factors arising from hyperlipidaemia and atherosclerosis are on rise despite a battery of drugs available in the therapy. So it is important to explore new potential targets for the treatment of hyperlipidemia apart from the already existing targets and therapy. These targets identified can be a useful in treatment of lipids either alone or in combination with each other. Based on these targets medicinal chemists around the world have been designing, synthesizing

& evaluating a variety of new molecules for antihyperlipidemic activity.

The review is a specifically focused and detailed account on the targets briefly discussed earlier as a subsection of a general review on hyperlipidemia by the corresponding author [297].

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Received: October 25, 2009 Revised: December 11, 2009 Accepted: December 12, 2009