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REVIEW ARTICLE

Recent developments in the treatment of atherosclerosis

PATHIK S. BRAHMKSHATRIYA, MITESH H. JANI, & MAHESH T. CHHABRIA

Department of Pharmaceutical Chemistry, L.M. College of Pharmacy, Navrangpura, Ahmedabad - 380009, Gujarat, India

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Abstract

Atherosclerosis is one of the most frequent causes of cardiac arrest. The major cause of this disease is high concentrations of lipid in the blood. Medicinal agents so far have been quite successful in the management of hyperlipidemia. Among the several widely used drugs, (fibrates, statins and niacin) statins are the most frequently prescribed in many forms of hyperlipidemia. Recently, statins have been found to produce serious toxicities, which are rare but can be potentially harmful and are noise concern for the immediate need to develop some new chemical entities in this category. This review is primarily concerned with recent developments in atherosclerotic drug discovery including novel inhibitors of cholesterol biosynthesis, cholesterol absorption inhibitors and antioxidants. The review also focuses on possible future targets including gene therapy.

Keywords: *Atherosclerosis, statins, coenzyme Q10, newer approaches, squalene epoxidase, squalene synthase*

Introduction

More than 40% of all deaths in the U.S. are from cardiovascular diseases (CVDs), and a person has a greater chance of dying from heart disease than from cancer, AIDS, diabetes and accidents combined. More than 2,600 Americans die each day due to CVDs—an average of 1 death every 33 seconds. One in 5 men and women has some form of CVDs. If all forms of major CVDs were eliminated, life expectancy would rise by almost 7 years [1]. CVDs are the major causes of death in adults in most developed and many developing countries, and are now the commonest cause of death worldwide [2]. These disorders also lead to substantial morbidity and disability and are a main source of the rising cost of health care. The most frequent cause of cardiac arrest is atherosclerosis. The total healthcare cost for atherosclerotic coronary heart disease (CHD) in 1997 was projected at $ 91 billion [3].

Risk factors for atherosclerosis

The effects of risk factors in adults are additive, the greater the number of high-risk factors present, the greater the risk of CVDs [4]. Cigarette smoking is a major risk factor for developing coronary artery disease (CAD) by producing a marked decline in endothelium-dependent vasomotor response [5–7]. Several studies suggest that exposure to environmental tobacco smoke (passive smoking) also increases the risk of developing heart disease [8–10]. It has been observed that exercise offers many benefits in CVDs [11]. People who exercise tend to live longer and have less risk of CVDs than those who do not [12]. Among adults, higher levels of physical activity are associated with a reduced incidence of CAD and hypertension [13]. Exercising vigorously is advantageous, but even moderate exercise has important protective effects [14]. It has been consistently reported that increasing degrees of obesity are accompanied by greater rates of CVDs [15–17]. Obesity is an independent risk factor for major coronary events although hypercholesterolemia and metabolic syndrome are often associated with it [18–20]. Diabetes of any type is independently associated with increased coronary diseases [21]. Both Type-I and Type-II diabetics are highly vulnerable to cardiac abnormalities. Studies have shown that mortality in diabetic patients is higher than in non-diabetics [22]. Elevated cholesterol (**1**) levels (hypercholesterolemia) in early life play an important role in

Correspondence: P. S. Brahmkshatriya, Department of Pharmaceutical Chemistry, L.M. College of Pharmacy, Navrangpura, Ahmedabad 380009, Gujarat, India. E-mail: pathik26@yahoo.com

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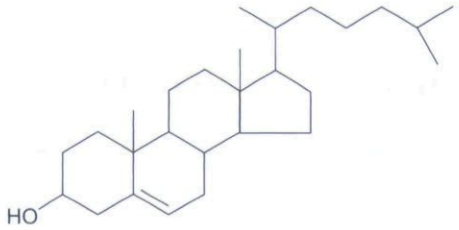
the development of adult atherosclerosis [23], and reducing cholesterol levels in children and adolescents will be beneficial in reducing the risk of atherosclerosis [24]. With as little as 1% decrease in total cholesterol levels, the incidence of CAD decreases by 2% [25]. A comprehensive review on novel risk factors for atherosclerosis has been published [26].



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LDL, HDL and atherogenesis

Cholesterol, being a hydrophobic molecule, is unable to travel as such in the aqueous plasma medium and lipoproteins serve as carrier molecules to transport cholesterol in various tissues of the body. Figure 1 represents a schematic diagram of a plasma lipoprotein.

Figure 2 represents a schematic representation of the transport of cholesterol in the tissues with the functions of various lipoproteins cited therein.

High cholesterol levels, more specifically, the low density lipoprotein (LDL) moiety, are well-recognized risk factors for CAD [27,28]. The protective properties and significance of high density lipoproteins (HDL) are difficult to comprehend without a clear understanding of the role of LDL cholesterol (LDL-C). Oxidized LDL is a major precursor of atherogenesis. Small amounts of oxidized LDL get trapped within the vascular wall and oxidation of LDL causes monocytes to bind to endothelial cells, which then migrate through the endothelial cells into the intima of the vessels. Once in the intima, monocytes become macrophages which engulf oxidized LDL. These LDL-laden macrophages are further oxidized converting into foam cells which accumulate within the intima of the artery and cause a proliferation of smooth-muscle cells resulting into fibrous cap of the atheroma [29]. Unlike LDL, HDL protects against atherogenesis through two mechanisms. First, HDL mediates the removal of excess cholesterol from peripheral tissues, such as blood vessels, and moves it back to the liver through a process known as Reverse Cholesterol Transport [30]. Once cholesterol is in the liver, it can be excreted from the body in bile. Therefore, higher levels of HDL allow excretion of excess cholesterol. Second, HDL impedes oxidation of LDL [29]. The Framingham Heart Study showed low HDL levels to be an independent risk factor for CAD, which showed 10% increase in CAD for each 4 mg/dL decrease in HDL [31]. Table I illustrates classification of lipids according to US guidelines [32].

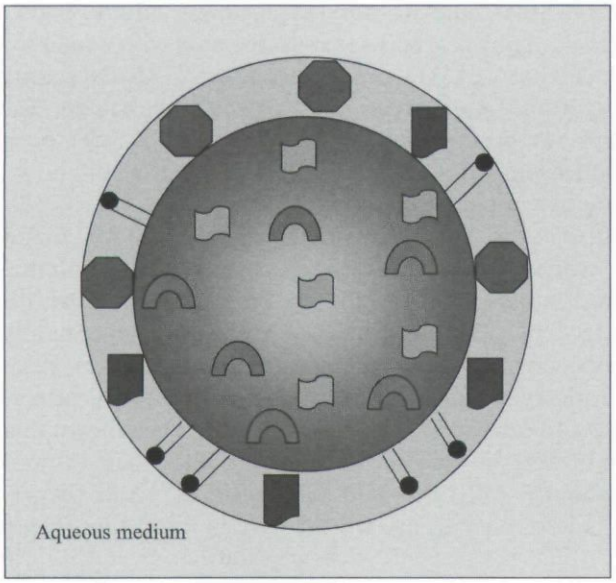


Figure 1. Schematic diagram of a plasma lipoprotein; - neutral core, - phospholipid molecule with polar head, - cholesterol, - apoprotein, - coat (shell), - cholesteryl ester, - triacyl glycerol

Management of hyperlipidemia

Dietary recommendations

The liver produces approximately 70% of the ~1 g of cholesterol utilized daily by a normal adult, the other 30% coming from dietary intake. Studies around the world indicate that fat and cholesterol are not the only nutrition concerns that relate to an attempt to prevent heart disease, but a low-fat, high-fiber diet rich in unrefined complex carbohydrates, helps to lower the risk of heart disease and improve heart health [33]. Reports have shown that fat rich in omega-3 fatty acids protects heart function [34,35]. Much attention has been devoted to the observation in some studies that moderate alcohol intake is associated with lower than average risk of CHD. However, it appears inappropriate to recommend the use of more than one alcoholic drink for cardioprotective purposes [36]. A 30–40% reduction in the incidence of heart disease was observed among those who had high vitamin E intake over a four to eight year period. The benefit seemed to be greatest in individuals taking 100–250 IU of supplemental vitamin E daily, which is a 6–15 times higher intake than the Recommended Dietary Allowances for vitamin E [37].

Drug therapy

There are many potent and effective drugs available nowadays with LDL lowering and HDL elevating properties for the treatment of atherosclerosis.

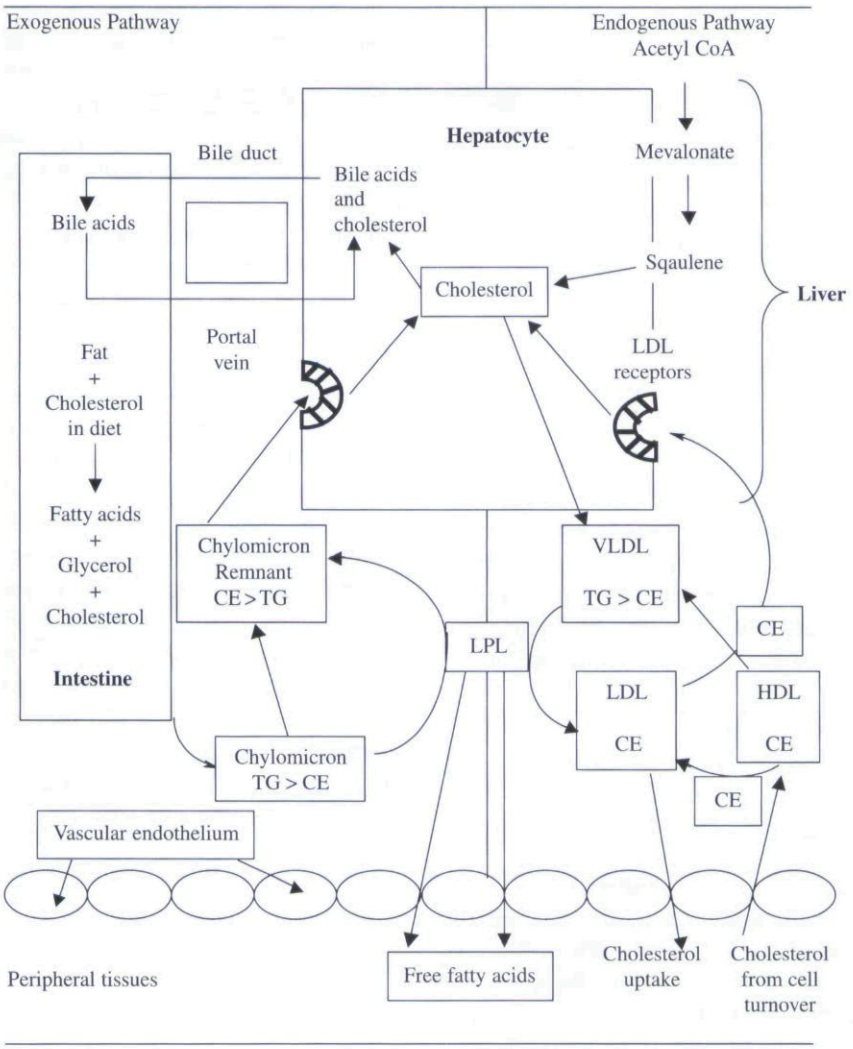


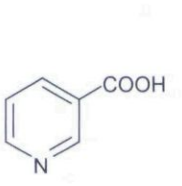
Figure 2. Schematic representation of the transport of cholesterol in the tissues; LPL—lipoprotein lipase, CE—cholesteryl ester, TG—triglycerides, LDL—low density lipoprotein, VLDL—very low density lipoprotein, HDL—high density lipoprotein.

Table I. Classification of lipid according to US guidelines.

Sr. No.	Lipid	Blood Concentration (mg/dL)	Risk factor for CAD
1	Total cholesterol	<200	Desirable
		200–239	Borderline high
		>240	High
		<100	Optimal
2	LDL-C	100–129	Near optimal/ above optimal
		130–159	Borderline high
		160–189	High
		≥190	Very High
3	HDL-C	<40	High
		≥60	Low
4	Triglycerides	<150	Normal
		150–199	Borderline high
		200–499	High
		≥500	Very High

(CAD—coronary artery disease, LDL-C—low density lipoprotein cholesterol, HDL-C—high density lipoprotein cholesterol)

Niacin. Niacin (**2**) is recommended for use only in primary hyperlipidemia (type IIa, IIb, III, IV, or V hyperlipoproteinemia) with a significant risk of coronary artery disease in those patients who have not responded to other measures alone.



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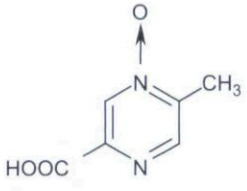

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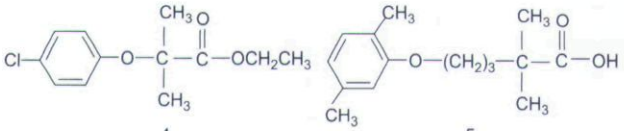
Efforts to dissect out untoward side effects have evolved the new analog acipimox (**3**), which is effective at lower doses with fewer side effects [38].

Fibrates. Fibric acids are employed primarily for the treatment of combined hypertriglyceridemia and

hypercholesterolemia. Clofibrate (**4**), gemfibrozil (**5**) and fenofibrate (**6**) are the commonly used fibrates.



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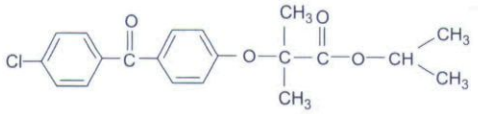

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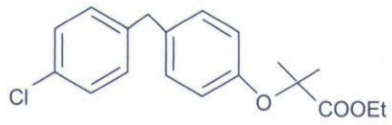
Fibric acids are thought to lower plasma triglyceride concentrations by stimulating the catabolism of triglyceride-rich lipoproteins (VLDL). This is exerted through an increased activity of the enzyme lipoprotein lipase, responsible for triglyceride hydrolysis in VLDL [39]. More active fibrates have been developed, most notably beclobrate (**7**), which is about nine times more potent than gemfibrozil [40].



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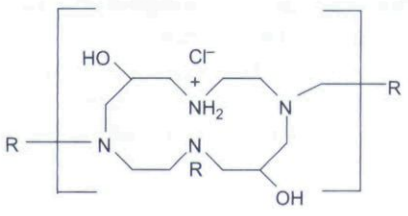
Bile acid sequestrants. Bile acid sequestrants are high molecular weight cationic ion exchange resins, which bind anionic bile acids in the intestine, thereby preventing their reabsorption in the liver. The consequence of the bile acid loss is a compensatory increase in cell-surface LDL receptors in the liver. This results in an enhanced uptake of LDL from plasma, with a reduction of LDL-C levels [41]. Colestipol (**8**) and cholestyramine (**9**) are the usually prescribed drugs in this category.



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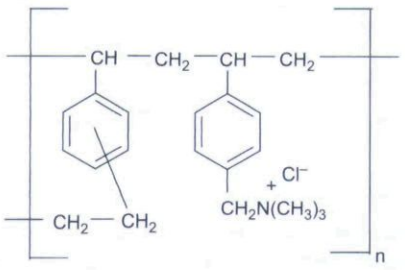

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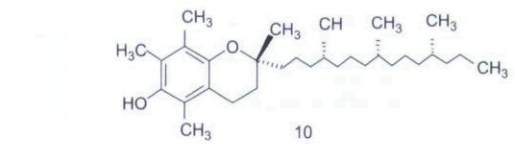
Antioxidants. It is now widely accepted that oxidative modification of LDL accentuates its atherogenicity by a variety of mechanisms [42] and that agents acting to inhibit this process may retard the atherosclerotic process [43,44]. This view is supported by studies with the antioxidants vitamin E (**10**) [45] and probucol (**11**), which clearly demonstrated their variety of antiatherogenic effects, which is supported by epidemiological data linking CHD with susceptibility of LDL to oxidation [46]. The search for more potent antioxidants has primarily uncovered compounds similar to probucol or vitamin E, i.e. lipophilic hindered phenols. Noteworthy are the spirocycle (**12**), which was 10–100 times as potent as probucol against LDL oxidation by Cu^{+2} or endothelial cells [47] and the tocopherol analog (**13**), which reduced serum cholesterol in a dose-dependent manner in mice [48]. A 71% reduction in arterial lesion area in cholesterol-fed rabbits was observed upon administration of *N,N'*-diphenyl-*p*-phenylenediamine (**14**), accompanied by significant protection of the LDL from oxidation. [49] Phenothiazine (**15**) was approximately ten times more potent than probucol at blocking Cu^{+2}-catalysed oxidation of LDL [50].



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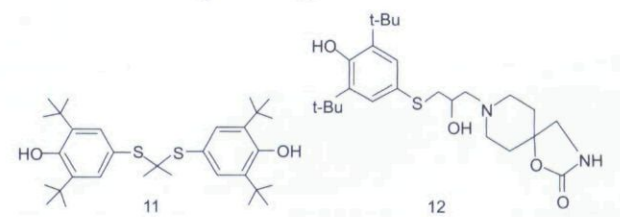

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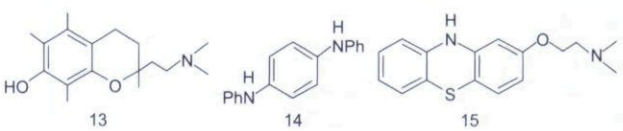

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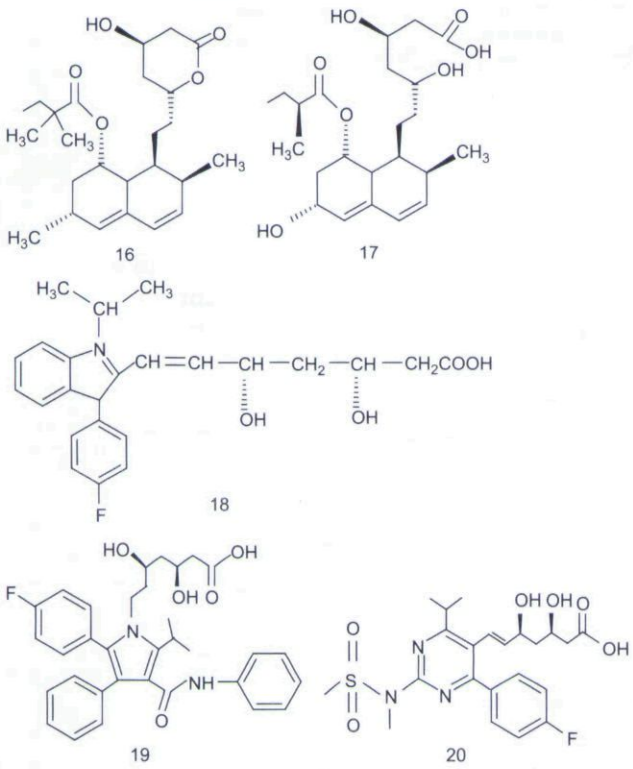
HMG Co A reductase inhibitors (statins). Statins are the most effective and best tolerated agents for treating dyslipidemia. These drugs viz. simvastatin (**16**), pravastatin (**17**), fluvastatin (**18**), atorvastatin (**19**) and rosuvastatin (**20**) are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, which catalyses an early rate limiting step in cholesterol biosynthesis (Figure 3).



Clinical trials have documented the efficacy and safety (see later) of statins in reducing fatal and nonfatal coronary events, strokes and total mortality [51–55]. Statins are also effective when given in combination with other drugs such as colestipol, cholestyramine [56] and fibrates [57]. Triple therapy with resins, niacin and statins can reduce LDL-C by up to 70% [58].

Coenzyme Q10

Coenzyme Q10 (Ubiquinone, CoQ10) is a natural, fat-soluble nutrient present in virtually every cell of the body. CoQ10 is the coenzyme for mitochondrial enzyme complexes involved in oxidative phosphorylation in the production of ATP [59–61].

Heart cells have a greater number of mitochondria, and subsequently, more CoQ10 than any other type of cells [62,63]. CoQ10 is essential for all cellular ATP production and is of particular importance in heart muscle function due to the tissue's extreme energy requirements. A deficiency of CoQ10 in the blood and the heart muscle has been documented in congestive heart failure (CHF) [64,65]. The second fundamental property of CoQ10 involves its antioxidant (free radical scavenging) functions [66,67]. CoQ10 is the only known naturally occurring lipid soluble antioxidant for which the body has enzyme systems capable of regenerating the active reduced ubiquinol form [68]. CoQ10 is carried in the blood with LDL and serves to diminish the oxidation of LDL-C in settings of oxidative stress [69]. CoQ10 is known to be closely linked to Vitamin E and serves to regenerate the reduced (active) α-tocopherol form of Vitamin E [70] as well as the reduced form of ascorbate [71]. Other more recently discovered aspects of CoQ10 functions include its involvement in extra mitochondrial electron transfer such as plasma membrane oxidoreductase activity [67], involvement in cytosolic glycolysis [72], and potential activity in both golgi apparatus and lysosomes [73]. CoQ10 also plays a role in improvement in membrane fluidity [74]. The multiple biochemical functions of CoQ10 have been reviewed [75]. CoQ10 is beneficial in treating and preventing CVDs and conditions such as high blood pressure [76], atherosclerosis [77], angina [78], and CHF [79]. It has been shown that heart attacks tend to occur when CoQ10 levels are low in the body [65]. Moreover, CoQ10 is beneficial against diabetes [80], immune dysfunction [81], cancer [82], periodontal disease [83], prostate cancer [84] and neurological diseases [85]. CoQ10 has been studied for its ability to improve the health of individuals with Parkinson's disease [85]. The exogenous administration of CoQ10 may play a positive role in the treatment of the loss or reduction of sperm motility in semen (asthenozoospermia) [86]. CoQ10 can significantly reduce the number of migraine attacks [87]. The presence of supplemental CoQ10 is a key to the heart's optimum performance [65].

CoQ10 depletion by statins

Statins have been shown to deplete the body of CoQ10 [88]. In a double-blind trial, individuals with high cholesterol who were treated with lovastatin or pravastatin for 18 weeks had a significant reduction in blood levels of CoQ10 [89]. Statins used to treat elevated blood cholesterol levels by blocking cholesterol biosynthesis also block CoQ10 biosynthesis [90]. The resulting lowering of blood CoQ10 level is due to the partially shared biosynthetic pathway of CoQ10 and cholesterol (Figure 3) [91]. Thus, this reduction would constitute a new risk of cardiac disease, since it is established that CoQ10 is indispensable for cardiac function [92]. Statin-induced CoQ10 deficiency is completely preventable with supplemental CoQ10 [92,93] with no adverse impact on cholesterol lowering [93] or anti-inflammatory properties of the statin drug [94].

Side effects of statins

Concurrent inhibition of CoQ10 is the prime reason for statin-induced rare but potentially severe side effects. Frequent side effects are no doubt a major reason why up to 75% of people taking statins discontinue their use [95]. *Myopathy* [96,67] and

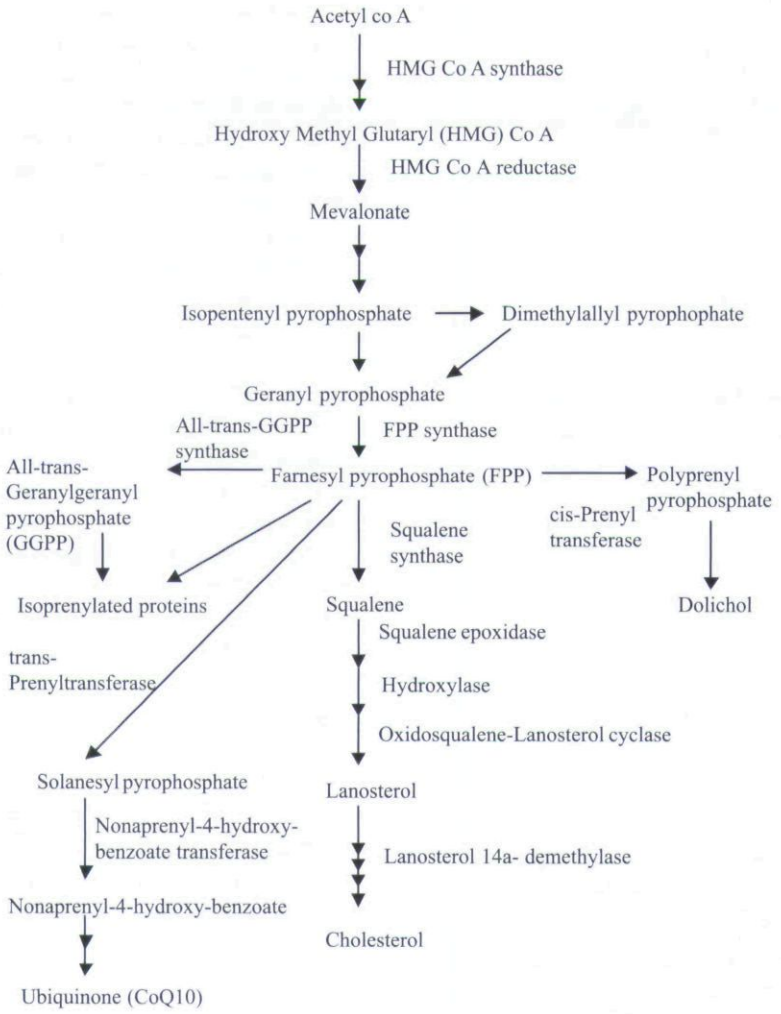


Figure 3. The Mevalonic Acid Pathway of Cholesterol Biosynthesis.

Rhabdomyolysis [98,99] are the most frequent side effects caused by statins.

Myopathy is a general term referring to any disease of muscles such as muscular dystrophies. General symptoms of myopathy include weakness of limbs, usually proximal (located closer to the center of the body) [100].

Baycol (cerivastatin) was withdrawn from the market by Bayer, after at least fifty-two deaths linked to the drug. Baycol was causing rhabdomyolysis, a condition characterized by severe muscle damage. This rare disorder occurs when a large number of skeletal muscle cells die, subsequently releasing massive amounts of myoglobin (a muscle protein) into the bloodstream which saturates the kidneys, effectively overwhelming their filtration capacities. Indeed, kidney failure was reportedly a major cause of death amongst the Baycol victims. Baycol is not unique in its ability to damage muscles but all the statins have been shown to produce muscle disorders in susceptible patients, and muscle pain is one of the most common reasons for patients being taken off statin drugs [101]. Physicians typically diagnose rhabdomyolysis and myopathy from statin drugs by means of a blood test that measures levels of the muscle protein called creatine kinase (CK). Researchers recently reported that some patients may suffer muscle deterioration caused by statins while still maintaining normal levels of CK [102].

Due to inhibition of myocardial ubiquinone supply, statins may also cause *cardiomyopathy* (a disease of the heart muscle). Ironically, while statins reduce the risk of atherosclerotic heart disease, their CoQ10-robbing effects have been linked to an increased risk of CHF. Figures from the National Center for Health Statistics show that since the early nineties, several years after statin drugs were available on pharmacy shelves, the incidence of CHF has increased sharply [103].

Statins are reported to cause *cancer* in rodents, in some cases at levels of animal exposure close to those prescribed to humans [104,105]. Statins are also reported to cause *peripheral neuropathy* [106–109] and *tendinopathy* [110]. Peripheral neuropathy is

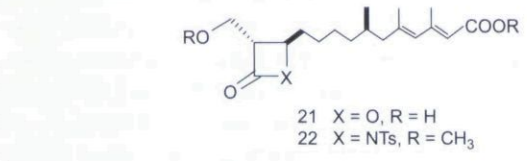
a neurological disorder resulting from damage to the peripheral nerves [111]. Tendinopathy is a term used to collectively describe tendinitis (tendon inflammation) and tendinosis (a slower-healing series of microtears to the tendon) [112].

Newer approaches to treatment of atherosclerosis

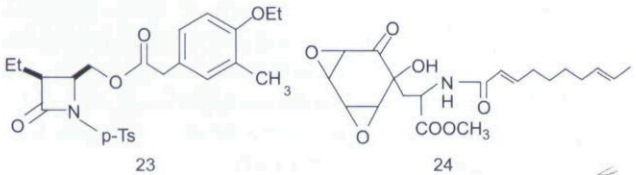
The undisputed success of current therapy for hypercholesterolemia, particularly the statins, has not deterred interest in discovering novel methods to reduce serum cholesterol or retard the progression of atherosclerosis. The majority of work has focused on inhibiting cholesterol biosynthesis at points other than HMG CoA reductase (HR) and on altering the absorption of dietary cholesterol. Following are newer approaches for the treatment of hyperlipidemia and atherosclerosis.

HMG CoA synthase (HS) inhibitors

HS catalyses the cholesterol biosynthetic step just prior to the reduction of HMG CoA (Figure 3). Reports have shown L-659,699 (**21**) as a potential HS inhibitor [113]. Structure Activity Relationship (SAR) work on (**21**) established that manipulation of the lipophilic side chain [114] is detrimental to activity, but the β-lactone can be substituted with an *N*-*p*-tosyl β-lactam (**22**) [115]. Subsequent analogous work identified (**23**) as one of the most potent of these tosyl lactams with an IC_{50} value of 2.1 nM [116]. A second class of inhibitors was recently isolated from fermentation broths; the most active component (**24**) had a modest IC_{50} value of 0.18 μM [117].



21 X = O, R = H
22 X = NTs, R = CH_3



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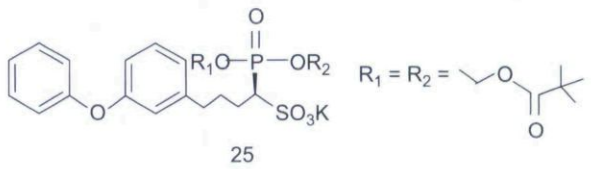
Squalene synthase (SS) inhibitors

The majority of efforts have focused on inhibition of SS, the first step committed in the biosynthesis of cholesterol (Figure 3). The substrate for the enzyme, farnesyl pyrophosphate (FPP), is water soluble and easily metabolized, thereby potentially avoiding harmful effects due to accumulation of the substrate during inhibition. A potent inhibitor of SS is BMS-188,494 (**25**), which is a prodrug having the ability to lower cholesterol in rats after oral administration, a model insensitive to lipid lowering with statins [118,119].



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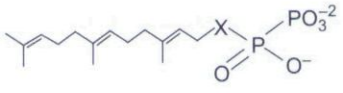
SAR studies within a series of stable FPP analogs resulted in the potent competitive SS inhibitor (**26**) (IC_{50} = 0.05 μM), which proved to be two orders of magnitude more active than the closely-related compound (**27**), illustrating the importance of the ether oxygen for efficient binding to the enzyme [120]. Azabicyclo[2.2.2]octane (**28**) inhibits rat liver microsomal SS with an IC_{50} of 11 nM in the presence of pyrophosphate [121], but it belongs to series of compounds also reported to be 5-HT_3 antagonists, causing doubt on its clinical potential. A group of fungal metabolites was recently isolated that exhibits extremely potent inhibition of SS. [122] Due to their virtually simultaneous discovery by two different groups, they are known collectively as either zaragozic acids or squalestatins. Zaragozic acid C (**29**) has an IC_{50} value of 9 nM against rat liver SS [123]. Administration of the closely related analog squalestatin 1 (**30**) subcutaneously to mice results in inhibition of hepatic cholesterol synthesis with an ED_{50} of 0.2 mg/kg [124,125].



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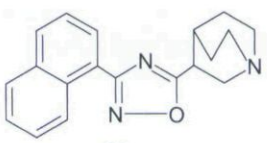
27 X = CH_2CH_2



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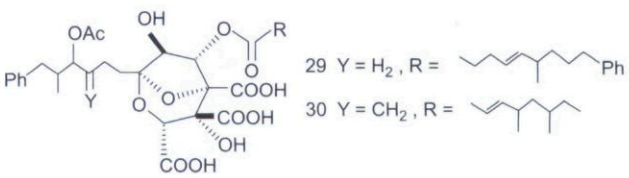
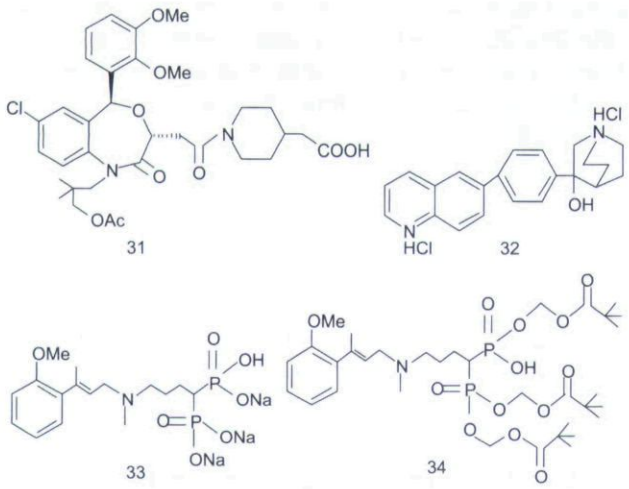
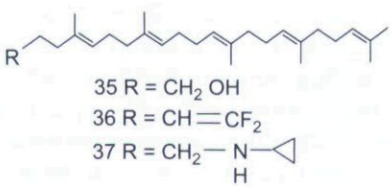


Lipid lowering properties of TAK-475 (**31**) have been examined [126,127]. Other novel SS inhibitors with potential hypocholesterolemic properties include RPR-107393 (**32**) [128], ER-28448 (**33**) [129,130] and ER- 27856 (**34**) [129,130].

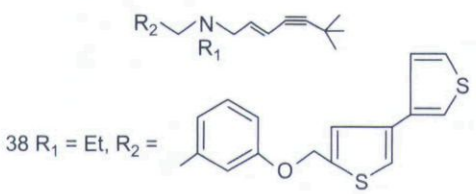


Squalene epoxidase (SE) inhibitors

This enzyme catalyses another rate-determining step in cholesterol synthesis, viz. Squalene epoxide from squalene (Figure 3). Trinorsqualene alcohol (**35**) was one of the first squalenomimetics to effectively inhibit SE ($IC_{50} = 4\mu M$) [131]. The 1,1-difluorosqualene (**36**) is orally active in mice, as indicated by dose-dependent reductions in hepatic cholesterol synthesis [132]. Roughly equivalent *in vitro* potency is seen with cyclopropylamine (**37**) against rat hepatic SE [133].

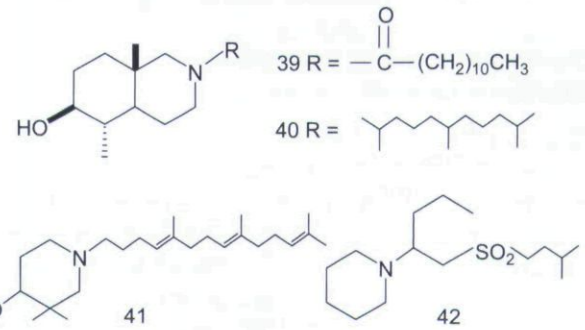


NB-598 (**38**), having an IC_{50} of 0.75 nM against HepG2 SE [134], effectively reduces serum cholesterol level and increases serum squalene in dogs following oral administration [135].

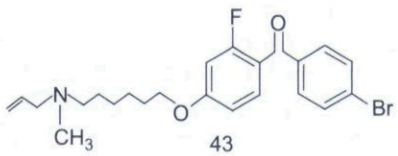


Oxidosqualene-lanosterol cyclase (SLC) inhibitors

The physiological substrate for SLC, 2,3-oxidosqualene, has served as the template for a number of synthetic inhibitors (Figure 3). Strong inhibition is obtained even with an amide such as azadecalin (**39**) ($IC_{50} = 0.7\ \mu M$) comparable to the older bicyclic amine (**40**) [136]. Roughly equivalent activity is observed with the monocyclic analog (**41**) also [137]. The piperidine sulfone (**42**) retains a significant hepatic SLC inhibition ($IC_{50} = 5\mu M$) within a structure markedly simpler than the squalene analogs [138].

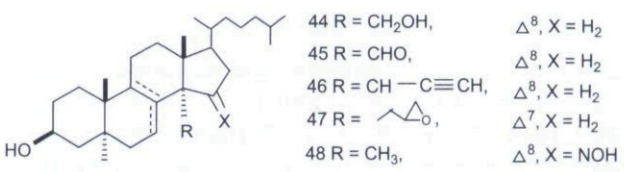


Another new SLC inhibitor, Ro 48–8.071 (**43**) has shown effective lowering of plasma cholesterol in hamsters and squirrel monkeys when compared to simvastatin [139].



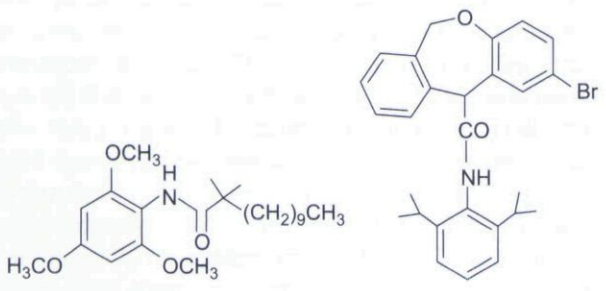
Lanosterol 14α-demethylase (LDM) inhibitors

Inhibition of the cytochrome P-450 enzyme LDM has the potential to attenuate cholesterol biosynthesis by a dual mechanism—direct blockage of the conversion of lanosterol to cholesterol (Figure 3) and indirect inhibition via a putative feedback mechanism whereby the accumulation of oxylanosterol intermediates (**44** and **45**) regulates the expression of HR [140]. The ethenyl lanosterol (**45**) is reported to function as an irreversible inhibitor of rat liver LDM [141] while the epoxide (**47**) is a competitive inhibitor with a K_i of 0.6 μM [142]. Lanosterols bearing substitution at the 15-carbon have been targeted due to their expected resistance to elimination of the 14-methyl group as formic acid, the final transformation induced by LDM. The oxime (**48**) is a modest inhibitor of LDM ($IC_{50} = 55\mu M$), but is an effective oral hypocholesterolemic agent in hamsters [143].



Acyl-Coenzyme A: Cholesterol acyltransferase (ACAT) inhibitors

One possible therapy of current interest is prevention of the absorption of dietary cholesterol by inhibiting the enzyme ACAT, which catalyses the intracellular esterification of cholesterol with fatty acids. It is now widely accepted that ACAT plays a key role in both the absorption of dietary cholesterol and in the accumulation of cholesterol within arterial tissue, two processes that are intimately involved in the atherogenic process [144]. Current research is strongly focused on developing inhibitors that act directly at the artery to determine if lesion development can be retarded [145]. ACAT inhibitors have the potential to function as antiatherogenic as well as hypocholesterolemic agents [146–148]. Cl-976 (**49**) decreases foam cell formation and cholesterol ester content in mechanically-induced arterial lesions in micropigs at a dose which does not alter plasma LDL-C or HDL-C levels [149]. KF-17828 (**50**) is reported to accelerate the regression of hypercholesterolemia in previously cholesterol-fed hamsters, implying a systemic effect more profound than simple withdrawal of dietary cholesterol [150]. Cl-1011 (Avasimibe) (**51**) has distinguished itself from all other agents by demonstrating cholesterol lowering in non-cholesterol fed animal models, a finding that may hold clinical significance as no other ACAT inhibitor has shown efficacy in this model [151].



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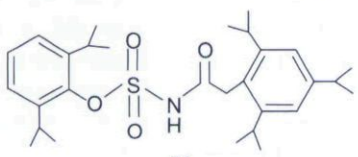

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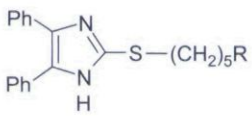
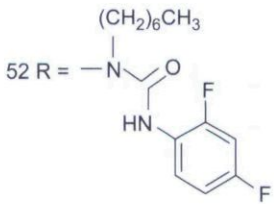
Some of the most effective members of this class, which are currently in clinical development, are Cl-976 (**49**) [152], DuP-128 (**52**) [153,154], RP-70676 (**53**) [155], YM17E (**54**) [156,157], and 447C88 (**55**) [158]. An extensive review on selective ACAT inhibitors as promising antihyperlipidemic, antiatherosclerotic and anti-Alzheimer drugs has recently been published [159].





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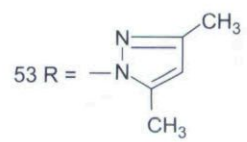

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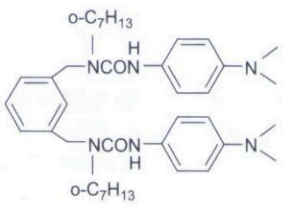

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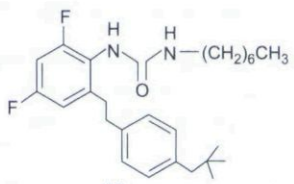

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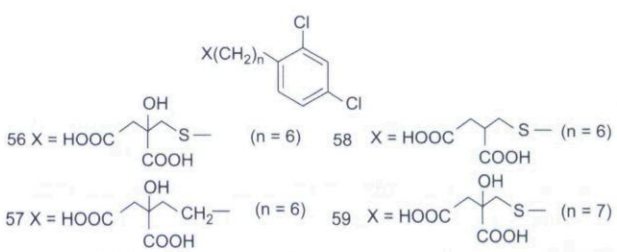
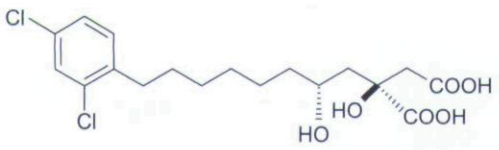
ATP-citrate lyase inhibitors

Mammalian ATP-citrate lyase is the main enzyme responsible for the supply of acetyl-CoA for synthetic pathways. The enzyme is present in most tissues but particularly in those with an active *de novo* synthesis of fatty acids such as adipose tissue and liver, especially during conditions of carbohydrate surplus. ATP-citrate lyase is the only enzyme shared by the synthetic pathways of fatty acid and cholesterol synthesis. Due to this unique position, it has been proposed that inhibition of this enzyme may be more efficacious in correcting mixed hyperlipidemia than statins [160]. A series of 2-substituted butanedioic acids have been designed and synthesized as inhibitors of the enzyme among which (**56–59**) are the most potent compounds [161].



Efforts to discover more potent analogs acting through this mechanism have uncovered compounds

SB-201076 (**60**) ($K_i = 1\,\mu M$) and its γ-lactone prodrug SB-204990 (**61**) [162].



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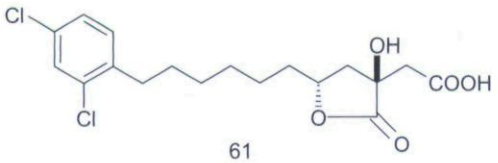

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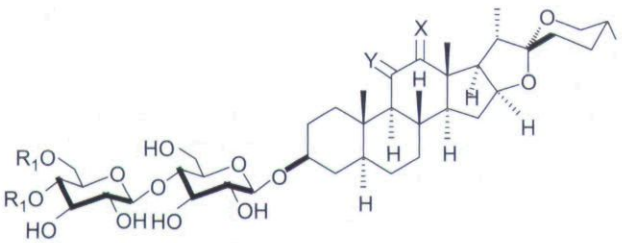
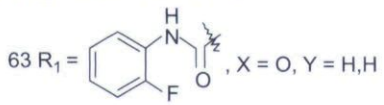
Cholesterol/Lipid absorption inhibitors

In addition to inhibiting cholesterol biosynthesis, reducing dietary cholesterol intake by inhibiting absorption at the intestinal wall exists as an alternative method of reducing LDL-C. Several agents, which inhibit cholesterol absorption and reduce LDL-C in animal models through an unknown mechanism of action, have been reported to possess modest clinical efficacy. The synthetic plant saponin derivative pamaqueside (CP—148,623) (**62**) [163] inhibited cholesterol absorption by 35–40% in normolipidemic individuals with a resulting 10–12% decrease in LDL-C at 300 mg twice daily [164]. SAR studies indicated that modifications at the 4′- and 6′- positions of the sugar moiety of (**62**) resulted in analogs, exemplified by CP-242,184 (**63**), which are 50–100 times more potent in the cholesterol-fed hamster model [165].

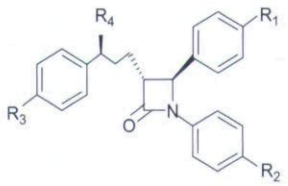


62 R_1 = H, X = H,H, Y = O



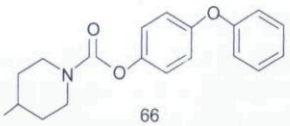
Azetidinone (SCH 48461) (**64**) was also reported to reduce plasma cholesterol in man [166,167]. Originally designed as ACAT inhibitors, *in vivo* activities of analogs from this series showed no correlation to ACAT inhibition but appear to be acting at the intestinal wall through an unknown mechanism [168]. In an effort to identify a more potent compound, the metabolic lability of (64) was examined [169,170]. Based on these findings and SAR studies, SCH 58235 (Ezetimibe) (**65**) was identified which showed 50-fold greater potency than (**64**) in the cholesterol-fed hamster model, greater metabolic stability and an improved pharmacokinetic profile [171]. Phase I/II clinical trials with (**65**) proved it as a novel selective cholesterol absorption inhibitor [172].

Despite controversy over its overall impact on cholesterol absorption, cholesterol ester hydrolase (CEH) inhibition remains of some interest [144]. WAY-121898 (**66**) reportedly inhibits CEH with IC_{50} value of 0.2 μM and reduces absorption of a single dose of cholesterol in normal-fed rats with an ED_{50} of 10 mg/kg [173]. Lipid lowering property of (**66**) through inhibition of pancreatic CEH has been reported [174].



64 $R_1 = R_2 = OCH_3$, $R_3 = R_4 = H$

65 $R_1 = R_4 = OH$, $R = R_3 = F_2$



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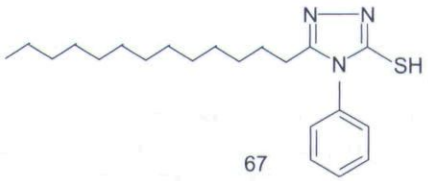
Cholesteryl ester transfer protein (CETP) inhibitors

CETP is a plasma glycoprotein that mediates the exchange of cholesteryl ester (CE) in HDL for triglyceride (TG) LDL [173,174]. This process decreases anti-atherogenic HDL-C and increases pro-atherogenic VLDL-C and LDL-C, so CETP is a potentially atherogenic protein. Although a possible anti-atherogenic role of CETP has also been suggested because of its participation in reverse cholesterol transport [175–178] many studies have supported its atherogenicity [179–184]. Therefore, an effective and safe CETP inhibitor may have potential as a novel anti-atherogenic drug and may be beneficial for patients with atherosclerosis and coronary heart disease. Reports have shown that the triazole (PD140195) (**67**) could selectively inhibit CE transfer in a non-competitive manner ($IC_{50} = 30\mu M$), while it did not inhibit TG transfer [185].



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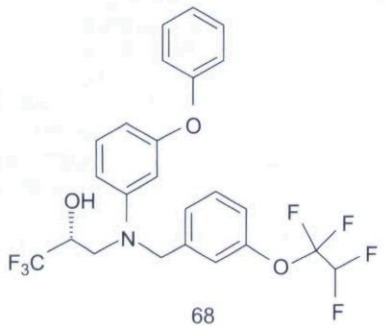
A trifluoro-3-amino-2-propanol (SC-795) (**68**) was also reported, and this agent inhibits CETP-mediated transfer of [3H] CE from HDL to LDL in buffer ($IC_{50} = 0.02\,\mu M$) and in human plasma ($IC_{50} = 0.6\,\mu M$) [186,187,188].



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Peroxisome proliferator-activated receptor (PPAR) Agonists

The PPARs are a subfamily of the 48-member nuclear-receptor superfamily [189] and regulate gene expression in response to ligand binding [190,191]. Various fatty acids serve as endogenous ligands for PPARs, whereas some members of the superfamily (farnesoid X receptors) bind bile acids and others (liver X receptors) bind oxysterols [189]. Three PPARs, designated PPARα, PPARδ (also known as PPARβ), and PPARγ, have been identified to date. PPARα is expressed predominantly in the liver, heart, and muscle, as well as in the vascular wall [191]. Fibrates such as fenofibrate, bezafibrate, ciprofibrate, and gemfibrozil act as full or partial PPARα agonists. In general, PPARα activation enhances free fatty acid oxidation, controls expression of multiple genes regulating lipoprotein concentrations, and has anti-inflammatory effects. PPARα agonists prevent or retard atherosclerosis in mice and humans [192,193]. Studies have shown that PPAR agonists can serve as very good candidates for antihyperlipidemic as well as antihyperglycemic activity [194].

Gene therapy

Several genes that are important in lipoprotein metabolism provide the opportunity for new therapeutic approaches. For example, a great deal is already known about the molecular mechanisms that regulate HR transcription [195] and degradation [196–198]. It is now quite easy to develop systems to discover drugs that specifically regulate gene transcription. Assays could be developed for any gene of interest in the cholesterol biosynthetic pathway. Such an assay has been developed for the HR gene [199]. Post-transcriptional down-regulation of HR has been reported with 15α-fluoro-lanost-7-en-3β-ol [200], 24(S),25-oxidolanosterol [201], and γ-tocotrienol [202]. Investigations to study the regulation of expression of HS [203], SS [204], and SE [205,206] are also underway. LDL receptors play a critical role in the catabolism of cholesterol, and a great deal is known about the molecular regulation of LDL receptor expression [207–209]. Therapeutic agents that stimulate the production of LDL receptors and thus lower serum LDL-C could be discovered in an assay employing the transcriptional regulatory elements from the LDL receptor gene [210]. Transgenic mice that overexpress the LDL receptor have lower plasma cholesterol concentrations than normal mice [211], and do not develop hypercholesterolemia when fed with a high-fat diet [212]. Apolipoprotein E (apo E) is a ligand for the LDL receptor and an important component of several lipoproteins [213–214]. Intravenous administration of apo E to rabbits causes a reduction in plasma cholesterol [215]. Severe hypercholesterolemia and atherosclerosis develop rapidly in mice that lack apo E [216,217]. Transgenic mice that overexpress apo E have low lipoprotein cholesterol levels and are protected from developing hypercholesterolemia when fed a high cholesterol diet [218]. These experimental observations suggest that increasing the expression of apo E could provide protection against atherosclerosis. Drugs that lower lipoprotein(a), a lipoprotein particle that has been shown to have a positive association with atherosclerosis [219–221], could be important for the prevention of atherosclerosis in those patients who have elevated levels of LDL-C. This strategy may be a useful and feasible form of gene therapy against atherosclerosis in humans.

Hormone replacement therapy (HRT)

After menopause, the rate of CAD in women rises sharply to match that in men. Both the Lipid Research Clinics Program Follow-up Study [222] and the 10-year follow-up to the Nurses' Health Study [223] showed that HRT reduced the risk of initial coronary events by about 40%. HRT is associated with favorable increases in HDL and decreases in LDL and total cholesterol levels [224]. HDL-C levels were increased at the most with use of estrogen alone; however, benefits were still seen when progestin was added to the estrogen regimen [225]. Postmenopausal women receiving conjugated equine estrogens (0.625 mg/day for 3 months) and medroxyprogesterone acetate (10 mg/day for 10 days after the study) showed an average 16% increase in HDL-C levels [226].

Conclusion

Significant efforts and progress have been made in recent years in the development of agents that lower

LDL-C levels. Future initiatives will undoubtedly be multifaceted, including HDL-C elevation, reducing the atherogenicity of various lipoproteins, and direct intervention at the arterial wall. Looking at some rare but potentially life threatening side effects of the most popular class of lipid lowering drugs, statins, the need for a more safer alternative is very crucial which will be as effective as statins. Out of the several possible newer approaches described, inhibitors of various enzymes involved in biosynthesis of cholesterol, viz. squalene synthase, oxidosqualene-lanosterol cyclase, squalene epoxidase, and lanosterol 14α-demethylase attract special attention. This is due to the fact that these agents inhibit cholesterol synthesis without concurrent inhibition of mevalonate, which is the precursor of CoQ10. This means of cholesterol synthesis inhibition without CoQ10 depletion will definitely minimize the toxicities associated with statins that are rare but can be potentially fatal. These agents are more likely to exert antihyperlipidemic activity comparable to that of statins.

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