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Review

The biology and chemistry of hyperlipidemia

Kishor S. Jain,[a,*] M. K. Kathiravan,[b] Rahul S. Somani[a] and Chamanlal J. Shishoo[c]

[a]*Sinhgad College of Pharmacy, S. No. 44/1, Vadgaon(Bk.), Sinhgad Road, Pune 411 041, India*
[b]*Poona College of Pharmacy, Pune 411 038, India*
[c]*L.M. College of Pharmacy, Ahmedabad 380 009, India*

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Abstract—Coronary arterial diseases are responsible for more deaths than all other associated causes combined. Elevated serum cholesterol levels leading to atherosclerosis can cause coronary heart disease (CHD). Reduction in serum cholesterol levels reduces the risk for CHD, substantially. Medicinal chemists all around the world have been designing, synthesizing, and evaluating a variety of new bioactive molecules for lowering lipid levels. This review summarizes the disorders associated with elevation of lipids in blood and the current strategies to control them. The emphasis has been laid in particular on the new potential biological targets and the possible treatments as well as the current ongoing research status in the field of lipid lowering agents.

Contents

Keywords: Cholesterol; Lipoproteins; Atherosclerosis; Antiatherogenic; Oxidation; Squalene synthase; Squalene epoxidase; Statins; Co-enzyme Q10.
* Corresponding author. Tel./fax: +91 20 24354720; mobile: +919422322070; e-mail: ks_jain@ vsnl.net

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1. Introduction

Today in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths.[1,2] In 1984 it was demonstrated for the first time that there exists a link between serum cholesterol levels and risk to coronary heart disease (CHD).[3] Worldwide, it causes deaths almost twice as many as those caused by cancer and 10 times as many as those caused by accidents. Despite significant medical advances, heart attacks due to coronary artery disease (due to atherosclerosis, that affects the arteries supplying blood to the heart) and stroke (due to atherosclerosis that affects the arteries supplying blood to the brain) are responsible for more deaths than all other causes combined.

A 1% drop in serum cholesterol reduces the risk for CHD by 2%.[4] In addition to this, different cholesterol lowering drugs or nonpharmacological treatments can significantly reduce morbidity from CHD, thus providing a causal role for cholesterol in coronary events.

2. Hyperlipidemia and atherosclerosis[5]

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease. A causal relationship between the elevated plasma lipids and the development of atherosclerotic plaques has been well established. Hyperlipidemia is an elevation of lipids in the bloodstream and these lipids include fats, fatty acids, cholesterol, cholesterol esters, phospholipids, and triglycerides. CHD is caused by the narrowing of the artery that supplies nutrients and oxygen to the heart. The main reason for this narrowing is atherosclerosis.

The word atherosclerosis is derived from the greek words, *athero* (meaning gruel or paste) and *sclerosis* (hardness). Atherosclerosis may be defined as degenerative changes in the intima of medium and large arteries. The degeneration includes accumulation of lipids, complex carbohydrates, blood and blood products, and cellular waste products, and is accompanied by the formation of fibrous

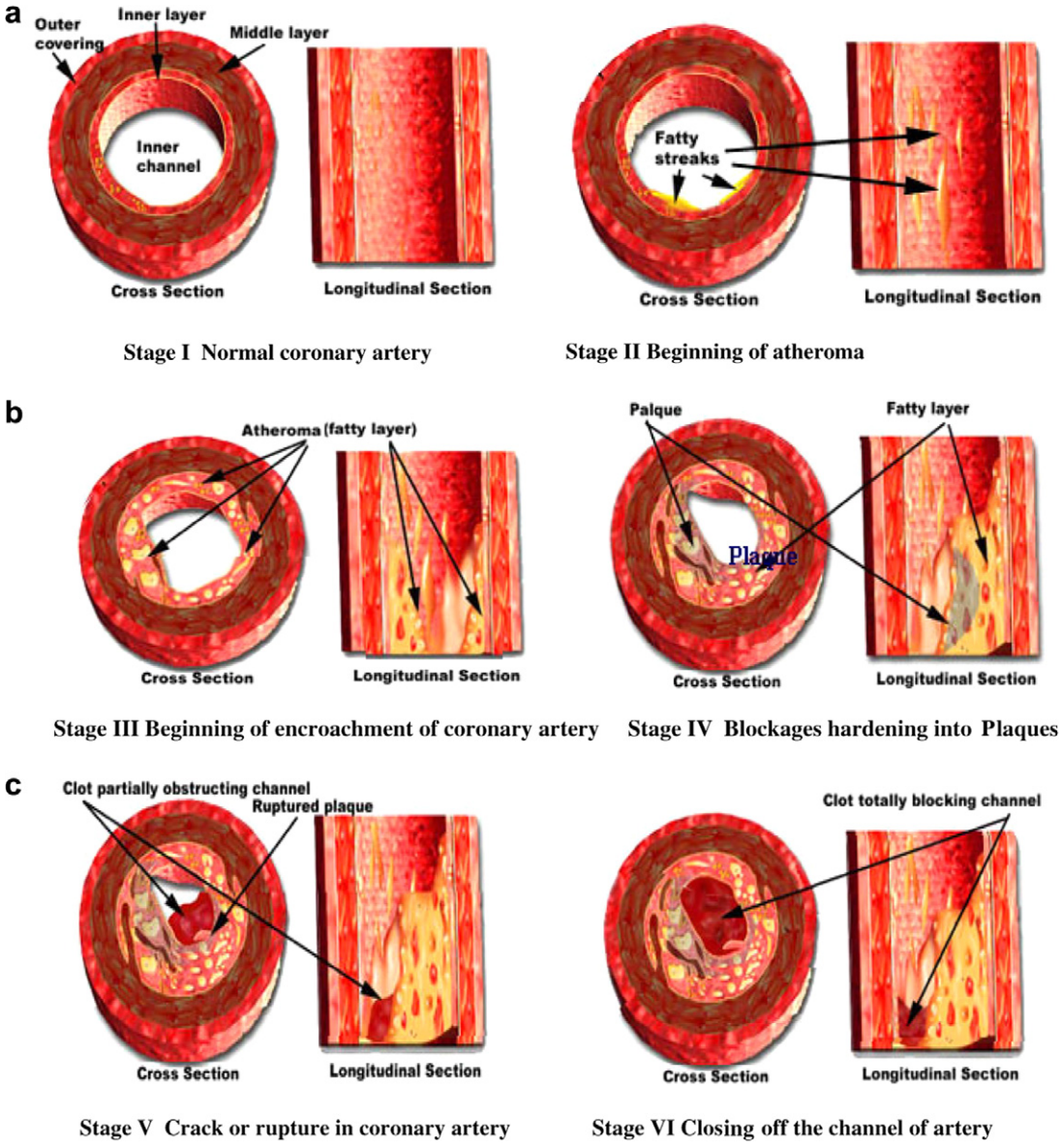


Figure 1. Different stages in progression of atherosclerosis.

tissues and calcium deposition in the intima of blood vessels. These deposits or plaques progressively decrease the lumen of the artery, reduce its elasticity, and may create foci for the thrombi and subsequent occlusion of blood vessels.

2.1. Different stages in progression of atherosclerosis[6]

Different stages of atherosclerosis from the normal, healthy coronary artery (stage I) until the final stage (stage VI) leading to its complete blockage culminating in heart attack are described in Figure 1a–c.

2.1.1. Stage I. The inner lining of the normal coronary artery is smooth and free of blockages or obstructions.

2.1.2. Stage II. However, with increasing age lipids or fatty substances (cholesterol and triglycerides) are deposited as fatty streaks which are only minimally raised and do not produce any obstruction or symptoms. This is just the beginning of atheroma.

2.1.3. Stage III. Further increase in builtup of fatty layers, atheroma, begins to encroach the inner channel which starts interfering with the free blood flow through coronary artery, thereby exposing the person to more risk of coronary artery disease.

2.1.4. Stage IV. With fibers beginning to grow in the fatty layers of the atheroma, the blockages harden into plaques, which increase the encroachment in the inner channels of the coronary artery. This encroachment may be upto 50% or more of its diameter and leads to obstruction sufficient to decrease the blood flow of heart muscle, even in the time of its increased need (exercise, emotional stress). This leads to elevation in blood pressure and heart rate.

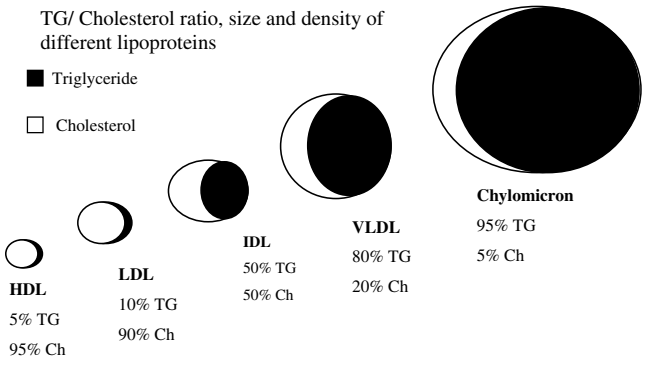


Figure 2. Lipoprotein classification.

2.1.5. Stage V. In some cases, plaques within the inner lining of the coronary artery may develop a slight crack or rupture, which stimulates the production of blood clots. The clots also get into the crack and cause it to rise and further obstruct the channel of the artery. The supply of the blood flow to the heart muscle is substantially reduced and the patient begins to have severe and prolonged chest pain that occurs at rest. This is known as unstable angina.

2.1.6. Stage VI. In case the clot does not fully close the channel of the artery and sufficient blood flow is maintained to the heart muscle, a heart attack may not develop, provided appropriate and prompt treatment is effected. However, the clot may continue to grow in many cases. This can completely fill the open channel of the artery and cutoff blood flow to the part of the heart muscle to which it is supplying.

2.2. Major families of blood (plasma) lipoproteins[7–12]

It is imperative here to understand in brief the various constituents often collectively referred to as lipids. These include fatty substances, fatty acids, cholesterol, triglycerides as well as lipoproteins, which are the combinations of the later two. The lipoprotein classification based on triglyceride and cholesterol is represented in Figure 2. Apolipoproteins, proteins that carry lipids in plasma, are also important, Table 1 briefly summarizes their composition and roles.

2.3. Apolipoproteins[13]

Apolipoproteins are the proteins that carry lipids in plasma; making the lipoproteins soluble in plasma and thereby acting as interface between plasma and the core components. They also play important role in stabilizing the structure of protein and act as ligands for specific receptors, having physiological role in lipoprotein metabolism. Table 2 summarizes the apoprotein classification and functions.

Table 1. The constitution, composition, and role of lipids

S.No.	Constituent	Composition	Effect/role
1.	Lipids		
	(a) *Simple lipids*	Saturated fatty acids (SFAs) e.g., Lauric acid	Raise blood cholesterol levels-harmful for health
		Monounsaturated fatty acids (MUFAs) e.g., Oleic acid	Reduce blood cholesterol levels-Raise HDL levels-good for health
		Poly unsatured fatty acids (PUFAS) e.g., Linolenic acid	Good for health
	(b) *Compound lipids*	Trans fatty acids (TFAs) e.g., Elaidic acid	Raise LDL cholesterol levels and lower HDL cholesterol levels harmful for health
	(c) *Neutral lipids*	Esters of fatty acids with alcohol along with phospholipids, sulfolipids, aminolipids, cerebrosides Uncharged lipids, glycerides, cholesterol and cholesterol esters	Harmful for health
2.	Cholesterol	Steroidal substances, essential components of cell membranes, brain and nerve cells, as well as bile. Useful in biosynthesis of vit. D and various sterol hormones	Higher levels can cause hypercholesterolemia; which is a risk factor
3.	Triglycerides (TG)	Major constituents of chylomicron and VLDL	Are energy substrates for liver and peripheral tissues, especially, muscles. High levels of TG is a risk factor
4.	Lipoproteins		
	Chylomicrons	95% TG and 5% Cholesterol	Mobilize dietary lipids, deliver dietary triglycerides to adipose tissues, muscles and dietary cholesterol to liver
	VLDL	80% TG and 20% Cholesterol	Transport triglycerols to extra hepatic tissues
	IDL	50% TG and 50% Cholesterol	They are either converted to LDL or taken up by the liver
	LDL	10% TG and 90% Cholesterol	Principal plasma carriers of cholesterol for delivering to peripheral tissues
	HDL	5% TG 95% Cholesterol	The apo E in HDLs leads to an increase uptake of cholesterol and its catabolism by the liver to lower the levels of intracellular cholesterol
5.	Lipoprotein (a)	Is an apoprotein (apo) B-100 lipoprotein	High concentrations are associated with increased apo (a) secretion
6.	Apoproteins	These are proteins that carry lipids in plasma, hence making lipoproteins soluble	They stabilize the structure of protein and act as ligands for specific receptors having physiological role in the lipoprotein metabolism

Table 2. Apoprotein classification and functions

Apoprotein—[MW (Da)]	Lipoprotein association	Comments
apo A-I (29,016)	Chylomicrons, HDL	Major protein of HDL, activates Lecithin, Cholesterol AcylTransferase (LCAT)
apo A-II (17,400)	Chylomicrons, HDL	Primarily in HDL, enhances Hepatic Lipase activity
apo A-IV (46,000)	Chylomicrons and HDL	Present in triacylglycerol rich lipoproteins
apo B-48 (241,000)	Chylomicrons	Exclusively found in chylomicrons, derived from apo B-100 gene by RNA editing in intestinal epithelium; lacks the LDL receptor-binding domain of apo B-100
apo B-100 (513,000)	VLDL, IDL, and LDL	Major protein of LDL, binds to LDL receptor; one of the longest known proteins in humans
apo C-I (7600)	Chylomicrons, VLDL, IDL, and HDL	May also activate LCAT
apo C-II (8916)	Chylomicrons, VLDL, IDL, and HDL	Activates Lipoprotein Lipase
apo C-III (8750)	Chylomicrons, VLDL, IDL, and HDL	Inhibits Lipoprotein Lipase
apo D (33,000)	HDL	Closely associated with LCAT activation
Cholesterol Ester Transfer Protein(CETP)	HDL	Exclusively associated with HDL and cholesteryl ester transfers
apo E (34,000) (at least 3 alleles [E_2, E_3,E_4] each of which has multiple isoforms)	Chylomicron remnants, VLDL, IDL and HDL	Binds to LDL receptor, apo E_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 apo B-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

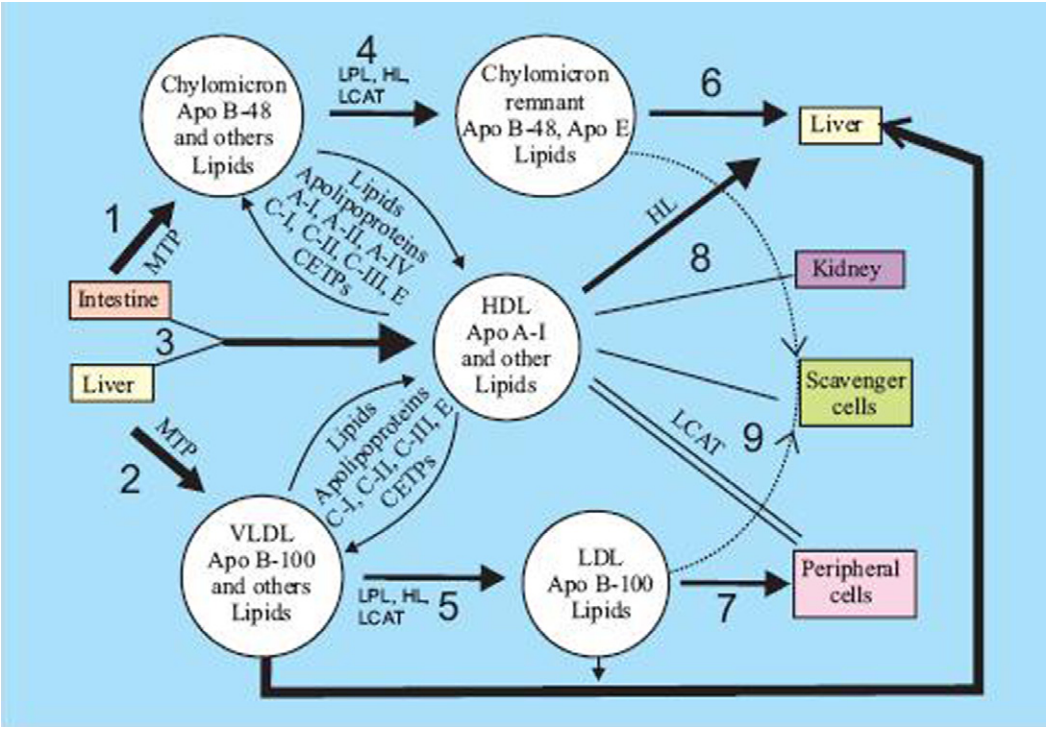


Figure 3. An overview of human apolipoprotein (apo) metabolism.

2.4. Pathways of lipid transport[14]

Cholesterol is absorbed from the intestine and transported to the liver by chylomicron remnants, which are taken up by the low-density lipoprotein (LDL)-receptor-related proteins (LRP). Hepatic cholesterol enters the circulation as very-low-density lipoprotein (VLDL) and is metabolized to remnant lipoproteins after lipoprotein lipase removes triglyceride. The remnant lipoproteins are removed by LDL receptors (LDL-R) or further metabolized to LDL and then removed by these receptors. Cholesterol is transported from peripheral cells to the liver by high-density lipoproteins (HDL). Cholesterol is recycled to LDL and VLDL by cholesterol-ester transport proteins (CETP) or is taken up in the liver by hepatic lipase. Cholesterol is excreted in bile. The points in this process are affected by the five primary lipoprotein disorders—familial hypertriglyceridemia (FHTG), familial

combined hyperlipidemia (FCHL), remnant removal disease (RRD) also known as familial dysbetalipoproteinemia, familial hypercholesterolemia (FH), and hypoalphalipoproteinemia.

3. Lipoprotein metabolism

The key organs that are involved in lipoprotein metabolism are intestine and liver.[15]Plasma apo B-48 concentration is regulated by secretion rates, that in turn are probably determined by the degree of intracellular degradation, which in turn is probably regulated by the amount of lipids in the enterocyte. After chylomicrons enter the bloodstream, they rapidly undergo lipolysis via the action of lipoprotein lipase and lose much of their triacylglycerols and acquire cholesteryl esters from other lipoproteins via the action of cholesterol ester transfer proteins (step 4) (Fig. 3). These remnants are taken up by the liver via receptors that bind apo E (step 6).

VLDL rapidly loses much of its triacylglycerol via lipolysis (step 5). LDL is cleared from plasma in part through the action of the LDL receptor (step 7) by both the liver and peripheral cells. Apo A-I is essential for HDL formation, because in its absence no HDL is present in plasma (step 3). Patients with familial hypoalphalipoproteinemia have decreased apo A-I production and premature CHD, whereas patients with familial combined hyperlipidemia or familial dyslipidemia (high triacylglycerol concentrations) have low HDL and apo A-I concentrations as a result of enhanced apo A-I fractional catabolism (steps 6 and 7). Patients with defects in cellular cholesterol efflux and defects in the ATP binding cassette protein A1 gene (*ABC A1* gene) have very small pre-HDL particles, marked hypercatabolism of apo A-I, and premature CHD (step 9).[16]

3.1. Enzymes modulating lipoprotein metabolism

3.1.1. Lipoprotein lipase.[17] Located on endothelial lining of arteries and capillaries and synthesized mainly in heart and skeletal muscles, it catalyzes the hydrolysis of various lipids on lipoprotein particles such as chylomicron and VLDL and turns them into smaller remnants that are rapidly cleared from blood stream. Apo C-II is necessary co-factor for its action.

3.1.2. Hepatic triglyceride lipase.[18] Located on hepatic endothelial cells, it catalyzes the hydrolysis of triglycerides, chylomicron remnants, and LDL, and phospholipolysis of HDL.

3.1.3. Lecithin cholesterol acyl transferase (LCAT).[19] This enzyme is synthesized in the liver and so named because it transfers a fatty acid from the C-2 position of

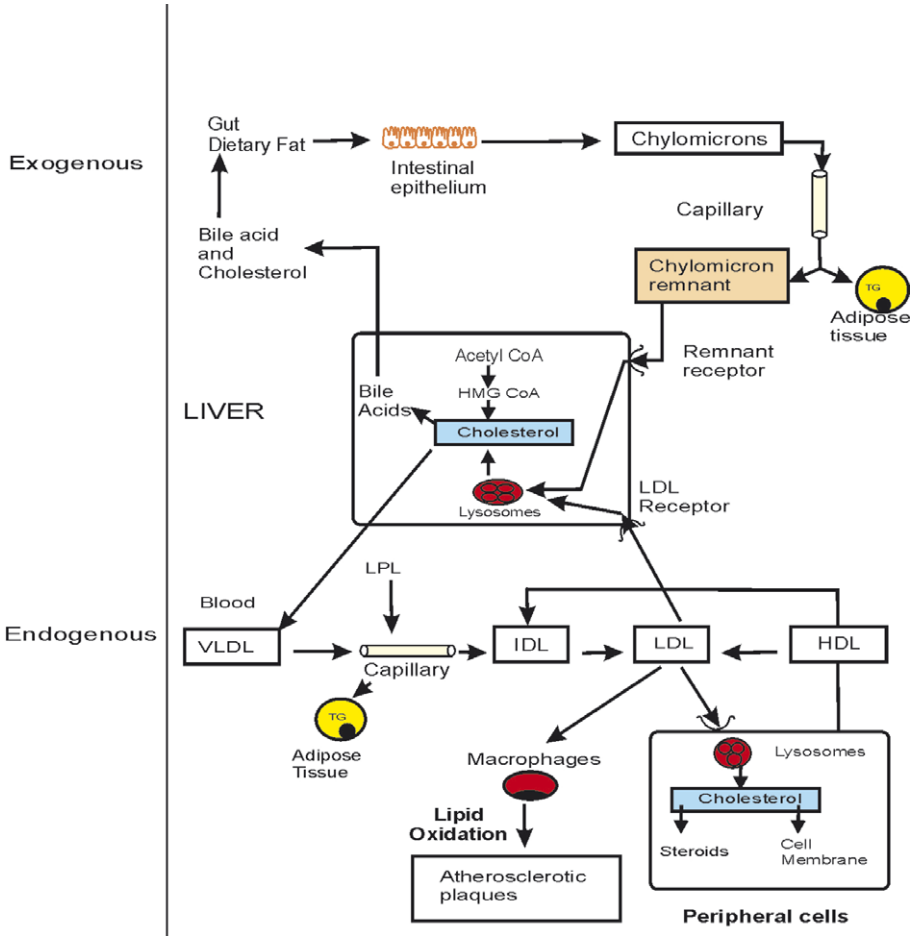


Figure 4. Exogenous and endogenous pathway of cholesterol.

lecithin to the C_3–OH of cholesterol, generating a cholesteryl ester and lysolecithin. The esters that are formed move into the core of HDL, enabling the HDL particles to acquire more free cholesterol from other lipoproteins and cell membranes. The activity of LCAT requires interaction with apo A-I, which is found on the surface of HDLs.

3.1.4. Cholesterol ester transfer protein (CETP).[20] The cholesterol esters of HDLs can also be transferred to VLDLs and LDLs through the action of cholesterol ester transfer protein. This has the added effect of allowing the excess cellular cholesterol to be returned to the liver through the LDL-receptor pathway as well as the HDL-receptor pathway.

3.1.5. Microsomal triglyceride protein (MTP).[21] It catalyzes the transfer of triglycerides, cholesteryl esters, and phosphatidylcholine between membranes and lipoproteins. It is a key protein in the assembly of apo B containing lipoproteins.

3.1.6. Acyl Co-A transferase (ACAT).[22] An endoplasmic reticulum-bound enzyme that catalyzes the formation of cholesteryl esters from cholesterol in a wide variety of cells. Cholesteryl esters are stored as cytoplasmic storage droplets or in the lipoprotein secreting cells.

4. Pathophysiology of hyperlipidemia[23]

An understanding of the biology of the lipoproteins and the pathophysiology of hyperlipidemic states is essential to the rational choice of treatment regimen.

4.1. Exogenous pathway: route of uptake of dietary lipids

Chylomicrons (CM) are complexes of triglycerides (TG), cholesteryl esters (CE), and apoproteins. After the removal of triglycerides they become chylomicron remnants (Fig. 4). Chylomicrons are degraded by lipoprotein lipase on endothelial cells of adipose tissue and muscle. After removal of TG for storage, the CM remnants are transported to the liver. This results in dietary TG stored in adipose tissue and muscles.

4.2. Endogenous pathway: route for distribution of cholesteryl esters (CE) from liver to target cells

VLDL is secreted by the liver into plasma and transported to adipose tissue and muscles, where lipoprotein lipase extracts most triglycerides. The remnant IDL is either taken up by the liver or circulated until the remaining triglycerides are removed forming LDL particles, rich in cholesterol. LDL is cleared from plasma through LDL receptor-mediated endocytosis. This results in transfer of TG from liver to target cells via VLDL, as well as, transfer of CE from liver to target cells via LDL (Fig. 4).

4.3. Route for cholesterol recovery[24]

Reverse cholesterol transport is a pathway where cholesterol is transported from atherosclerotic plaques or other lipids back to liver to be excreted into the faecus via bile. As cell dies and the cell membranes turnover, free cholesterol is released into the plasma. It is immediately absorbed into HDL particles, esterified with a long chain fatty acid by lecithin cholesterol acyl transferase (LCAT), and transferred to VLDL or IDL by a cholesteryl ester transfer protein in plasma. Eventually, it is taken up by the liver as IDL or LDL, thus resulting in the recovery of cholesterol from cell membranes and reincorporation into LDL pool or return to liver.

4.4. De novo cholesterol biosynthesis

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

4.5. Cholesterol excretion by enterohepatic circulation

Bile salts are synthesized from cholesterol in the liver, released into the intestine, and recycled. A small amount of bile acid is excreted. This results in conversion of liver cholesterol to bile salts for excretion.

5. Types of hyperlipoproteinemias (Fredrickson/WHO classification)[25]

The classification of hyperlipoproteinemias is presented in Table 3.

Type I: Severe elevation of chylomicrons and TG due to congenital deficiency of lipoprotein lipase or apo C-II. Deposition of fats in skin represents clinical manifestations of the disorder.

Type IIA: Elevation of LDL cholesterol. Genetic conditions responsible are familial hypercholesterolemias, polygenic hypercholesterolemias, familial combined hyperlipidemia, and familial defective apolipoprotein B 100. These individuals are at high risk of developing premature coronary artery disease.

Type IIB: Characterized by the elevation of both LDL cholesterol and triglyceride levels. Familial combined hyperlipidemia is the most common genetic cause of this disorder where both VLDL and LDL levels are elevated.

Type III: Develops due to a defect in VLDL remnant clearance. Also known as familial dysbetalipoproteinemia. These individuals have difficulty in removing triglyceride rich VLDL remnant particles and this consequently leads to elevation of cholesterol and triglycerides.

Type IV: Characterized by hypertriglyceridemia (250–500 mg/dl). Causes are multiple as well as genetic. Diseases contributing to this are diabetes, nephrosis along with administered medications.

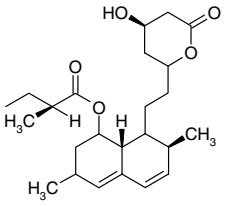
Type V: Elevated levels of chylomicrons and VLDL. Defective lipolysis and an overproduction of VLDL are responsible for this. Causes can be genetic or can

occur secondary to diabetes mellitus, obesity or alcohol consumption.

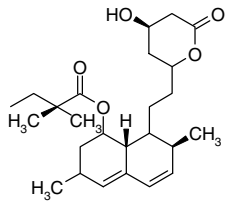
6. Treatment of hyperlipidemia

6.1. Hydroxymethyl glutarate coenzyme A (HMG-CoA) reductase inhibitors (statins)

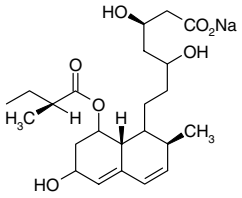
The statins (Lovastatin **1**, Simvastatin **2**, Pravastatin **3**, Fluvastatin **4**, Atorvastatin **5**, Rosuvastatin **6**, Pitavastatin **7**) competitively inhibit HMG-coenzyme A reductase, which is involved in the rate limiting step of cholesterol biosynthesis in the liver. In addition, statins increase levels of HDL which has cardiovascular protective effects. Furthermore, statins reduce the susceptibility of lipoproteins to oxidation, both in vitro and ex vivo.[26] Oxidative modification of LDL appears to play a key role in mediating the uptake of lipoprotein cholesterol by macrophages.



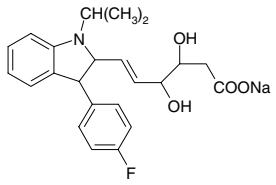
Lovastatin **1**



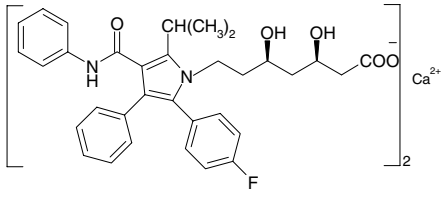
Simvastatin **2**



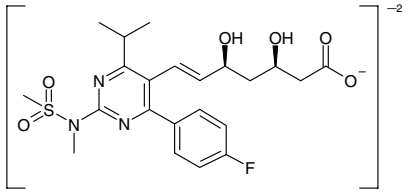
Pravastatin **3**



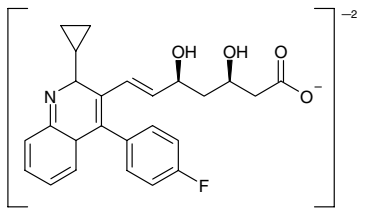
Fluvastatin **4**



Atorvastatin **5**



Rosuvastatin **6**



Pitavastatin **7**

6.1.1. HMG-CoA reductase chemistry.[27,28] HMG-CoA reductase (HMGR) catalyzes the rate-limiting step in cholesterol biosynthesis. The reduction of 3-hydroxy-3-methylglutaric acid (HMG) to revalonic acid involves the transfer of 4 electrons (via 2 molecules of NADPH cofactor) to a substrate that has been activated for reaction with the sulfhydryl (–SH) containing coenzyme A (designated as CoASH).

The binding site for the HMG-CoA substrate is of critical importance. Some of the key amino acid residues of HMGR that bind to the HMG-CoA substrate have been identified (Figs. 5 and 6).

The binding interactions of HMG-CoA reductase and its substrate include:

- Lys_{735} (in cationic conjugate acid form), which anchors the substrate to the enzyme through an ion–ion bond with the C_5 anionic carboxylate group of HMG-CoA.
- Lys_{692}, also in the cationic form, stabilizes the carbonyl oxygen of which group through an ion–dipole bond.
- The C_3-OH group of the substrate, which is stabilized by two residues, Ser_{684} and Asp_{690}. Serine acts as an H-donor to form a H-bond with the oxygen of the OH group, and anionic Asp forms an ion–dipole bond with the alcoholic hydrogen.
- Lys_{691} (in cationic conjugate acid form), engages in an ion–dipole bond with the carbonyl oxygen of C_1. It is this carbonyl group that gets reduced to the primary alcohol through the action of the HMGR enzyme. Lys_{691} is found in the region (or domain) of the receptor referred to as the *cis* loop.
- An anionic Asp_{767}, promotes this important ion–dipole interaction by forming its own ion–ion bond with Lys residue. This interaction stabilizes the action for interaction with the HMG-CoA substrate.
- Glu_{559} (in unionized acid form), also forms hydrogen bonds to the same C_1 carbonyl oxygen. By forming these two important bonds, this carbonyl group is held tightly to the enzyme, and is properly oriented for the reduction to occur.

In addition to these interactions, Tyr_{479} engages through van der Waals bond with the adenine base of the CoA portion of the substrate, forming a kind of 'hydrophobic shield' that closes down the binding pocket for effective reduction by the NADPH cofactor.

During the first NADPH reduction, the doubly bonded nitrogen of His_{866} (in cationic acid form) acts as a hydrogen donor to the sulfur atom of the thioester (SCoA), to liberate CoASH from the substrate. This, in turn, produces the mevaldehyde intermediate.

6.1.1.1. Side effects. Myopathy and rhabdomoylsis are the most frequent side effects of statins. Baycol® (cerivastatin) was withdrawn from the market by Bayer due to rhabdomoylsis. Kidney failure was reportedly a major cause amongst the Baycol® victims. Also due to

Table 3. Types of hyperlipoproteinemias

Type of Lipids	Type of hyperlipoproteinemia					
	I	IIa	IIb	III	IV	V
Cholesterol	N, >	≫	≫	N, ≫	N, >	N, ≫
Triglycerides	≫	N	≫	N, ≫	≫	≫
Lipoproteins						
Chylomicrons	≫	N	N	N	N	≫
VLDL	N, >	N, ≪	≪	N, >	≫	≫
ILDL				≫		
LDL	≪	≫	≫	≫	N, <	≪
HDL	≪	N	N	N	N, <	≪
Treatment	Diet	Diet, Statins, Bile Acid Seques-trants, Nicotinic Acid	Diet, Statins, Bile Acid Sequestrants, Fibrates, Nicotinic Acid	Diet, Fibrates, Nicotinic Acid	Diet, Fibrates, Nicotinic Acid	Diet, Fibrates, Nicotinic Acid

N, normal; >, slight increase; ≫, significant increase; <, slight decrease; ≪, significant decrease.

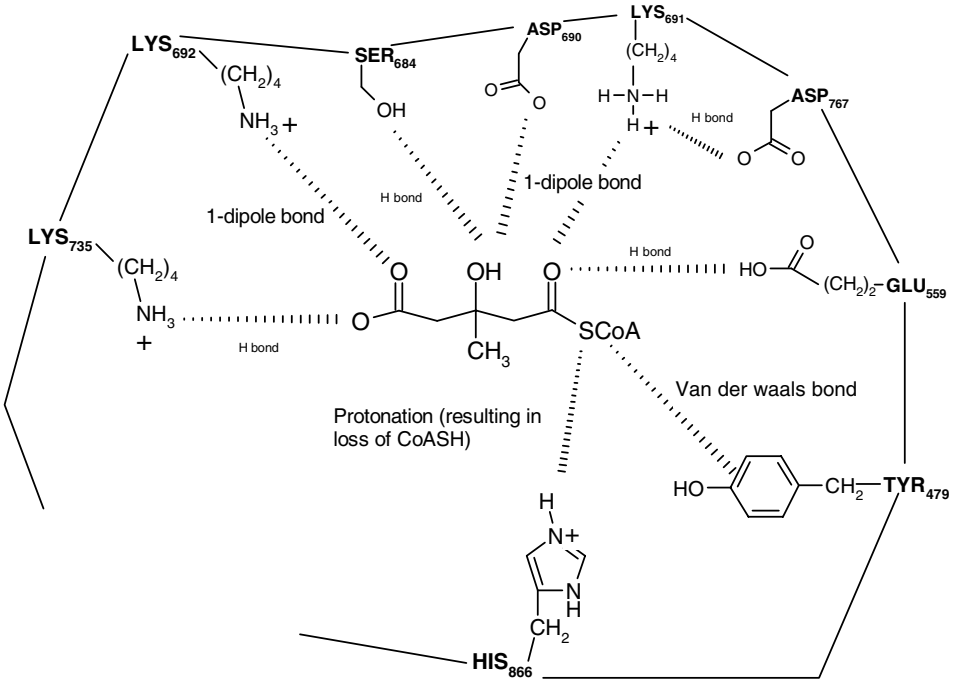
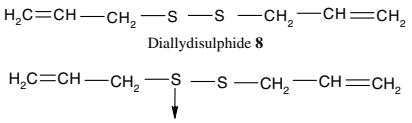


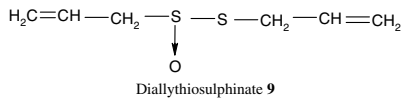
Figure 5. HMG-CoA reductase binding interactions with the endogenous HMG CoA Substrate. (Reprinted with permission from the American Journal of Pharmaceutical Education.[27])

inhibition of myocardial ubiquinone supply, statins may cause cardiomyopathy.

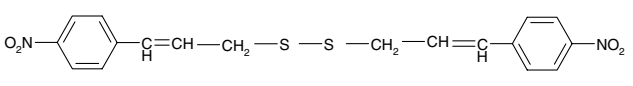
6.1.2. Other HMG-CoA reductase inhibitors. The lipid-soluble compound diallyldisulfide (DADS) **8**, found in garlic, has been shown to depress cholesterol synthesis by 10–25% at lower concentrations. Diallylthiosulfinate, a metabolite of allicin, **9** at a concentration of about 10 μM blocked the formation of 7-dehydrocholesterol and reduced the production of cholesterol. The derivatives with electron withdrawing substituents on the phenyl ring were found to lower hepatic cholesterol levels by 37% and inhibit the activity of HMG-CoA reductase by 56%. The derivatives with electron donating substituent on the phenyl ring showed weaker activity as evidenced by their reduced capacity to lower hepatic cholesterol levels and inhibit HMG-CoA reductase. Thus, certain novel derivatives of diallyldisulfide such as bis-(3-(4-nitrophenyl)prop-2-ene)disulfide **10** are effective in lowering cholesterol levels and could be potentially beneficial in treating hypercholesterolemia.[29,30]



Diallydisulphide **8**



Diallythiosulphinate **9**

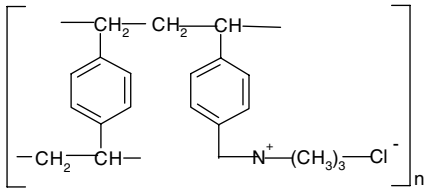


Bis(3(4-nitrophenyl)prop-2-enyl)disulfide **10**

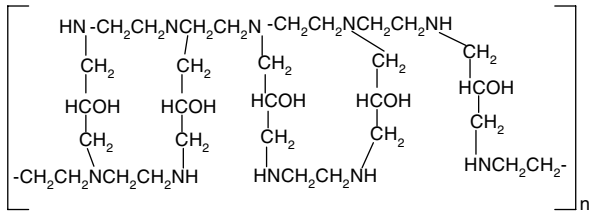
6.2. Bile acid sequestrants

Bile acid sequestrants (anion-exchange resins) are highly positively charged resins and bind to the negatively charged bile acids. Because of their large size, these resins are not absorbed and thus the bound bile acids are excreted in the stool. Consequently the liver's pool of bile acids depletes, leading to increased conversion of cholesterol to bile acid in hepatocytes. Decline in hepatic cholesterol content stimulates the production of LDL receptors and also increases synthesis of cholesterol in the liver. The increase in hepatic LDL receptors increases LDL clearance and lowers LDL-C levels. However this effect is partially offset by the enhanced cholesterol synthesis caused by the upregulation of HMG-CoA reductase.[31]

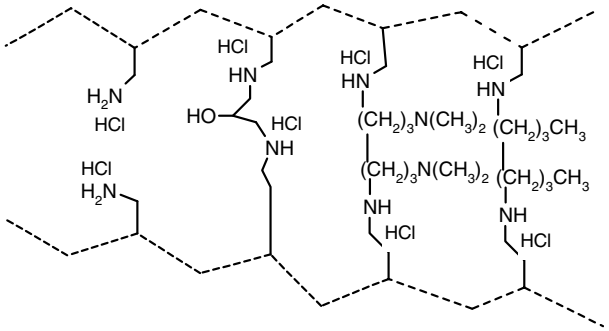
Cholestyramine **11** and Colestipol **12** are well-established bile-acid sequestrants. Cholestyramine a polymer of styrene and divinylbenzene with active sites formed from trimethylbenzylammonium groups is a quaternary amine. Colestipol, a co-polymer of diethylenetriamine and chloro-2,3-epoxypropane, is a mixture of tertiary and quaternary diamines.



Cholestyramine **11**



Colestipol **12**

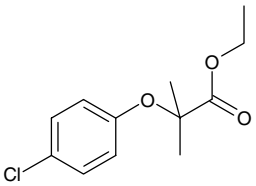


Colesevelam Hydrochloride 13

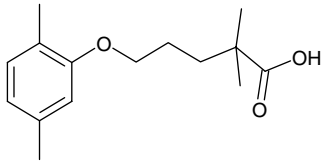
6.2.1. Side effects. Major side effects of these resins are constipation and osteoporosis on long-term therapy. They may exacerbate hypertriglyceridemia by an unknown mechanism. Recently, a novel drug, Colesevelam, with increased in vitro potency[32] showed lesser side effects. Colesevelam **13** may not cause dyspepsia, constipation and it does not exacerbate hypertriglyceridemia, considerably. Bile acid sequestering agents may contribute to calcium loss and therefore increase the risk for osteoporosis. Overtime deficiencies of vitamins A, D, E, and K may occur and vitamin supplements may be necessary.

6.3. Fibrates or lipoprotein lipase stimulants (fibric acid derivatives)

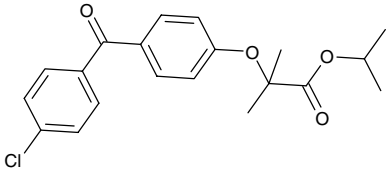
The fibrates (Clofibrate **14**, Gemfibrozil **15**, Fenofibrate **16**, Ciprofibrate**17**, and Benzafibrate **18**) affect lipid metabolism as agonists of the enzyme *peroxisome proliferator activated receptor alpha* (PPARα). This results in the peripheral lipolysis of triglyceride rich lipoproteins through the stimulation of lipoprotein lipase, a reduction in apoprotein C-III, and an increase in apoprotein A-I production.[33] Fibrate mediated increase in HDL-C is due to PPARα stimulation of apo A-I and apo A-II expression, which increases HDL levels.[34]



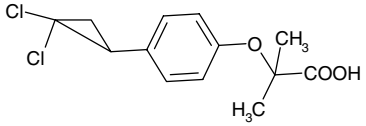
Clofibrate **14**



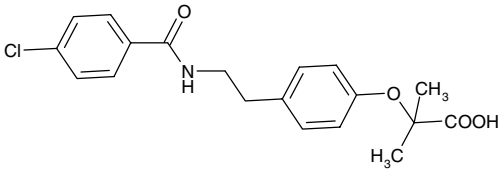
Gemfibrozil **15**



Fenofibrate **16**



Ciprofibrate **17**



Benzafibrate **18**

Though, the primary effect of fibrates is marked reduction in triglyceride levels, additionally moderate reduction in LDL cholesterol may also be seen. Four key mechanisms responsible for the effects of fibrates are; increased lipolysis, increased hepatic fatty acid uptake, reduced hepatic triglyceride production, and increased

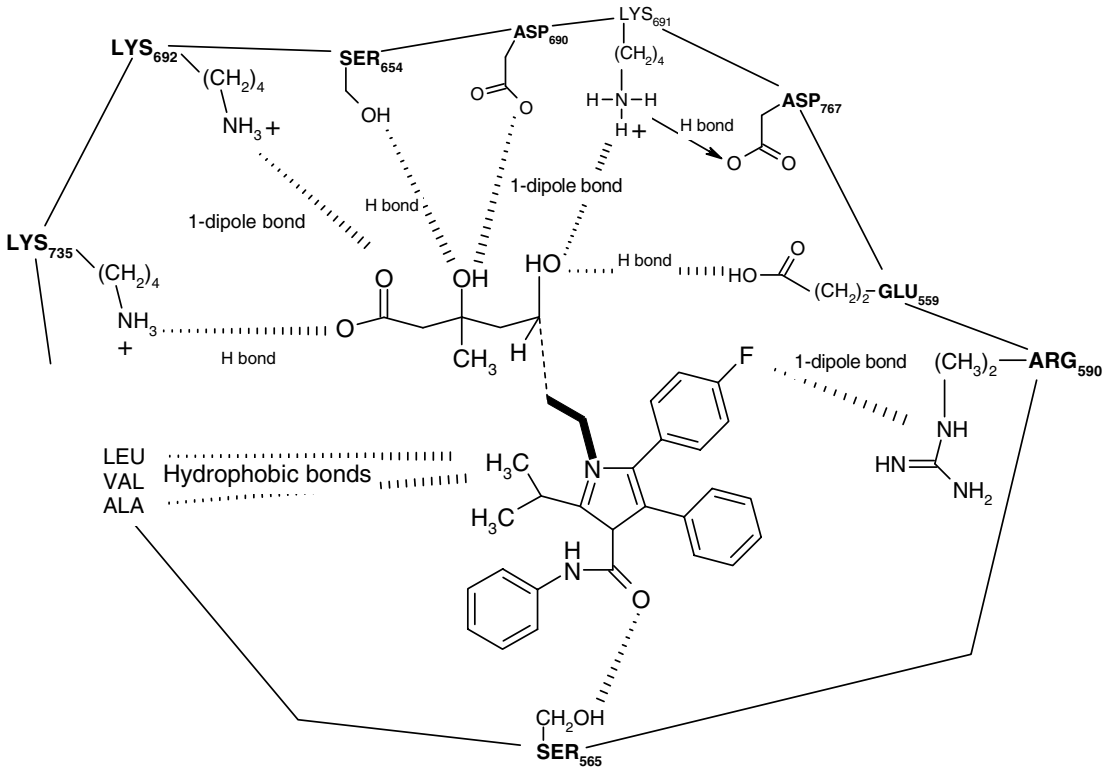


Figure 6. HMG-CoA reductase binding interactions with atorvastatin. (Reprinted with permission from the American Journal of Pharmaceutical Education.[27])

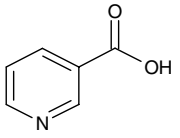
LDL uptake by LDL receptors. Stimulation of reverse cholesterol transport results in increased HDL.

Clofibrate (ethylester of *p*-chlorophenoxy*iso*butyrate) is the prototype of fibric acid derivatives.

6.3.1. Side effects. Side effects may include gastrointestinal discomfort, aching muscles, sensitivity to sunlight, and skin rashes. Fibrates have been known to cause gallstones. The drugs may cause abnormal heart rhythms and can affect the liver and kidney.

6.4. Niacin

Niacin **19** is a water-soluble vitamin of type B, used to treat dyslipidemia. Niacin inhibits the lipolysis of triglycerides by hormone-sensitive lipase, which reduces transport of free fatty acids to liver and decreases hepatic triglyceride synthesis. In liver niacin reduces triglyceride synthesis by inhibiting both the synthesis and esterification of fatty acids that increases apo B degradation.[35]



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Reduction of triglyceride synthesis reduces hepatic VLDL production, which accounts for the reduced LDL levels. Niacin also enhances lipoprotein lipase (LPL) activity, which promotes the clearance of chylomicrons and VLDL triglycerides. Niacin raises HDL-C levels by decreasing the fractional clearance of apo A-1 in HDL, rather than by enhancing HDL synthesis.[36]

6.4.1. Side effects. The most common side effects are flushing of the face and neck, itching, headache, blurred vision, and dizziness. Gastrointestinal problems are common.

6.4.2. Recent advances. Advicor^{R} a once daily combination of Niaspan (extended release niacin) and lovastatin lowers LDL and TGs to a greater extent than lovastatin alone and can raise HDL by as much as 40%.

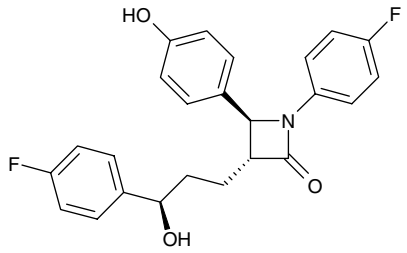
7. New potential targets and treatments

7.1. Cholesterol absorption inhibitors[37]

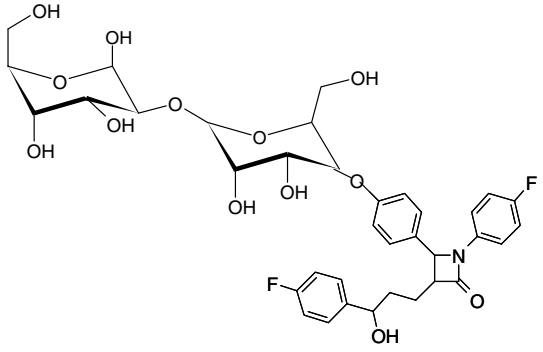
Absorption of cholesterol occurs in the proximal small intestine and requires emulsification, hydrolysis of ester bonds, micellar solubilization, absorption into the intestinal cells, reesterification within the cells, incorporation into chylomicrons, and secretion into intestinal lymphatics. Chylomicrons enter the blood from the lymph, where lipoprotein lipase hydrolyzes triglycerides, converting them into chylomicron remnants. Within the liver, the cholesterol delivered by chylomicrons enters the cholesterol pool that controls the synthesis of several key proteins in the regulation of cholesterol metabolism, including the LDL-C receptors and HMG-CoA reductase enzyme.

Ezetimibe **20** is a selective cholesterol absorption inhibitor that blocks a yet unidentified sterol transporter that moves cholesterol into the wall of the small intestine.[38]

It reduces LDL-C between 10 and 19% in monotherapy. Interestingly, the reduction of LDL-C in combination of ezetimibe with a statin (Simvastatin **2** or Atorvastatin **5**) is additive.



Ezetimibe **20**



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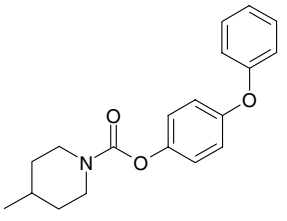
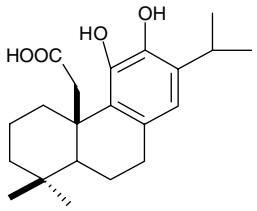
Recently, a wide range of nonhydrolyzable phenolic glycosides **21** of ezetimibe were synthesized and demonstrated to be active inhibitors of cholesterol absorption using brush border membrane vesicle assay. The analogues of azetidines provided access to a variety of inhibitors in vitro, suggesting that the lactam of ezetimibe merely serves as a ring scaffold to appropriately position the required substituents.[39]

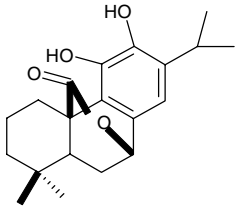
Compound WAY-121898 **22** reportedly inhibits cholesterol ester hydrolyses[40] with IC_{50} value of 0.2 M and reduces absorption of a single dose of cholesterol in normal fed rats with an ED_{50} of 10 mg/kg.[41]



WAY-121898 **22**



Carsonic acid **23**



Carnosol **24**

Carsonic acid **23** and carnosol **24**, a bietan type diterpene, were isolated from leaves of *S. officinalis* as a new class of pancreatic lipase inhibitors.[42] They substantially inhibited pancreatic lipase activity with IC_{50} values of 36 and 13 μM, respectively. Carsonic acid significantly inhibited triglyceride in olive oil loaded mice at a dose of 5–20 mg/kg, po.

7.2. Peroxisome proliferation activated receptor (PPAR) agonists

PPARs are a subfamily of the 48-member nuclear receptor super family[43] and regulate gene expression in response to ligand binding.[44,45] PPAR receptors are ligand dependent nuclear transcription factors that have been implicated in the regulation of lipids and glucose metabolism, morphogenesis, cell growth, differentiation, and homeostasis.

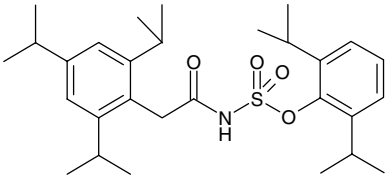
Three mammalian PPARs have been identified and are termed as PPAR-α, -γ, and -δ. PPARs regulate expression of target genes by binding to DNA sequence termed as PPAR response elements. The PPAR-γ receptor subtype is predominantly expressed in adipose tissue and plays a pivotal role in adipocyte differentiation, suggesting PPAR-γ is an important component in the adipogenic signaling cascade and in lipid storage and utilization. The human PPAR-γ gene structure has been characterized and two isoforms with a common ligand binding domain, PPAR-γ_1, and PPAR-γ_2 have been identified.

PPAR-α activation enhances free fatty acid oxidation, controls expression of multiple genes regulating lipoprotein concentration and anti-inflammatory effects.[46]

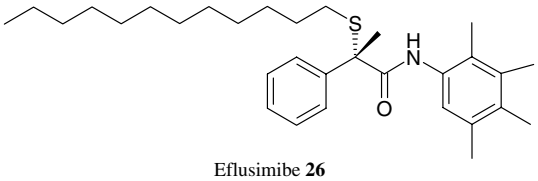
Li et al.[47] found PPAR-α and PPAR-γ ligands protective against atherosclerosis and to inhibit macrophages' foam cell formation. Studies have shown that PPAR agonists can serve as very good candidates for antihyperlipidemic, as well as, antihyperglycemic activity.

7.3. Acyl-CoA cholesterol acyl transferase inhibitors (ACAT)

Acyl-CoA cholesterol acyl transferase (ACAT) is an enzyme which has two isomeric forms, ACAT-1 and ACAT-2, and is responsible for cholesterol esterification in macrophages, liver, and intestine. Cholesterol ester formation by macrophages through the activity of ACAT-1 results in foam cell formation in artherosclerotic lesions. Therefore, ACAT-1 inhibitors have antiatherogenic effect.[48] In intestine ACAT-2 may reduce cholesterol absorption. Avasimibe **25** acts by inhibiting ACAT in the arterial wall thus slowing the development of atherosclerosis by several possible mechanisms. Another drug Eflucimibe **26** inhibits ACAT.[49]

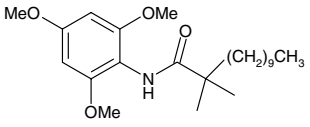


Avasimibe **25**

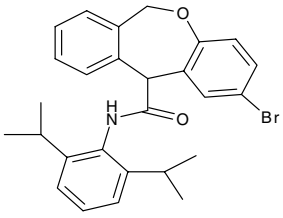


Eflusimibe **26**

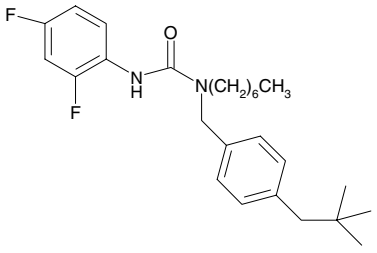
Many of the following compounds, **27**–**30**, have shown to inhibit ACAT activity in vitro.[50–53] The molecule KF-17828[51] **28** has been reported to accelerate the regression of hypercholesterolemia in cholesterol fed hamsters implying systemic effect more profound than simple withdrawal of dietary cholesterol. Some of the most effective members of this class, which are currently in clinical development, are compounds **31**, **32**.[54,55]



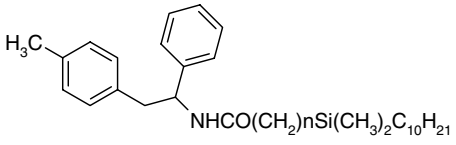
CI-976 **27**



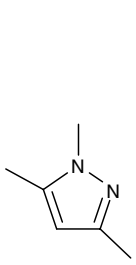
KF-17828 **28**



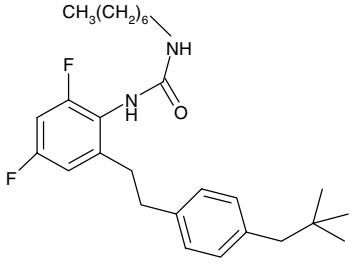
CL 277, 082 **29**



SA-58, 035 **30**



RP-70676 **31**



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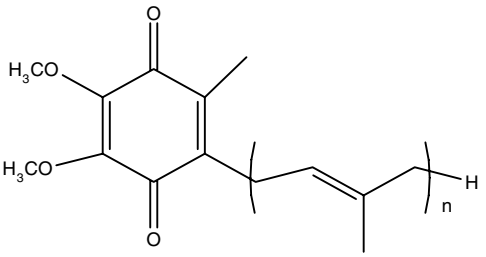
7.4. Coenzyme Q10 (CoQ10)

CoQ10 or ubiquinone **33** is an essential vitamin or vitamin-like substance. CoQ10 is the coenzyme for mitochondrial enzyme complexes involved in oxidative phosphorylation in the production of ATP.[56–58] A deficiency of CoQ10 in human heart disease has been documented.[59] The biosynthesis of CoQ10 is a complex 17-step process requiring atleast seven vitamins (vitamin B_2, riboflavin, vitamin B_3, niacin, Vitamin B_6, folic acid, vitamin B_{12}, and pantothenic acid) and several trace elements.



Coenzyme Q (n=6-10) **33**

HMG-CoA reductase inhibitors used to treat elevated blood cholesterol levels by blocking cholesterol biosynthesis also block CoQ10 synthesis.[60] The resulting lowering of blood pressure by CoQ10 is due to the partially shared biosynthetic pathway of CoQ10 and cholesterol.

CoQ10 is the only known naturally occurring lipid soluble antioxidant for which the body has enzyme reduced ubiquinol form. CoQ10 is carried in the blood with LDL and serves to diminish the oxidant of LDL-C settings of oxidative stress.[61]

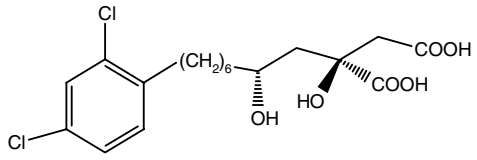
CoQ10 is the coenzyme for atleast three mitochondrial enzymes (complexes I, II, III), as well as, enzymes in other parts of the cell. CoQ10 is beneficial in treating and preventing CVDs and conditions such as high blood pressure,[62] atherosclerosis,[63] angina,[64] and CHF.[65]

7.5. ATP citrate lyase inhibitors

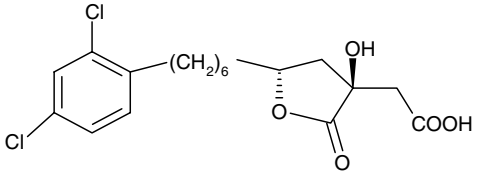
ATP citrate (*pro*-S)lyase is a cytosolic enzyme that catalyzes the cleavage of citrate into acetyl CoA and oxaloacetate.[66–68] In nonruminating mammals this enzyme is abundantly expressed in lipogenic tissues, such as the liver and adipose tissue,[69] where it has an important role in supplying acetyl CoA for both cholesterol and lipogenesis.

Studies with (−)hydroxycitrate, a potent inhibitor of ATP citrate lyase, demonstrated that inhibition of this enzyme leads to decrease in the synthesis of both cholesterol and fatty acids[70–74] and an increase in low-density lipoprotein (LDL) receptor activity.[75] 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.[74] Pearce et al.[76] identified a series of potent inhibitors of ATP citrate lyase such as SB-201076 **34**, having a k_i of 1 μM for the human enzyme. Also its prodrug lactone SB-204990 **35** inhibited cholesterol synthesis and fatty acid synthesis in Hep G2 cells (dose related inhibition up to 91% and 82%, respectively) and rats (76% and 39 %, respectively).

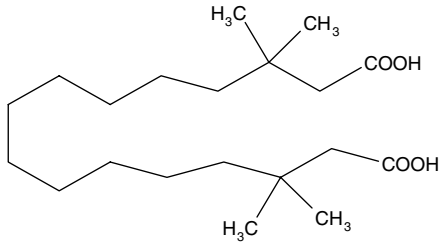


SB-201076 **34**



SB-204990 **35**

A series of 2-substituted butanedioic acids have been designed and synthesized as inhibitors of the enzyme.[77] Another compound, MEDICA 16 (M 5693) **36**, reduced plasma lipids dramatically.[78,79]



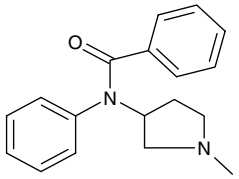
M 5693 **36**

7.6. Antiatherogenic agents (HDL enhancers)

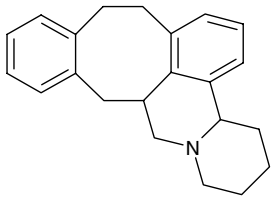
Angiographical studies have shown that elevated levels of some HDL particles appear to be correlated to a decrease in the number of sites of stenosis on the coronary arteries of humans.[80] HDL may protect against the progression of atherosclerosis through several mechanisms.[81]

In vitro study has shown that HDL is capable of removing cholesterol from cells. Data of this nature suggest that one 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.[82] In addition HDL may serve as a reservoir in the circulation for apoproteins necessary for the rapid metabolism of triglyceride rich lipoproteins. Accordingly, agents that increase serum HDL-C concentration would be of utility as antiatherosclerotic agents in the treatment of dyslipoproteinemia and related coronary heart diseases.

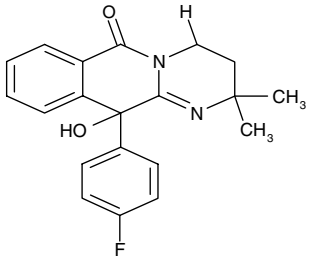
Data mining for compounds that enhance HDL properties led to the thio containing classes of compounds such as thiohydantoin, thiouracils, thiosemicarbazone, and sulfonyl imidazolidiones. The benzamide series **37** and taclamin **38** were amongst the nonthiocontaining hits. Based on the common structural elements of the nonthio containing compounds, a pharmacophore was generated and a 3D search of the database identified the novel tricyclic imidazoisoquinolone **39** series. Several derivates of this series exhibited potent HDL-C enhancing activity in animal models.[83]



Benzamide series **37**



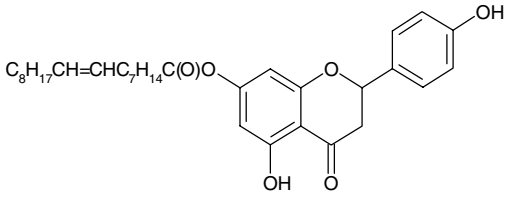
Taclamin **38**



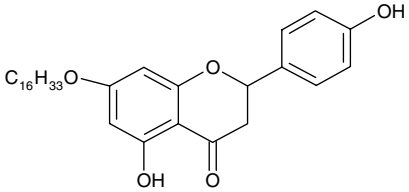
Tricyclic imidazoisoquinolone **39**

Naringenin, a compound from citrus flavonoids, is an effective antiatherogenic agent. Aglycone of naringenin was found to inhibit the formation of aortic atherosclerosis lesions in rabbits fed with high diet cholesterol.[84] They exhibited hypocholesteremic activities by reducing cholesterol biosynthesis and cholesterol esterification in high diet fed rats.

As potent anti-atherogenic agents two types of naringenin derivatives, ester **40** and ether **41**, were prepared and evaluated for aortic atherosclerotic lesions. The compounds significantly inhibited the formation of aortic fatty streaks in atherosclerotic high cholesterol diet fed rabbits.[85]

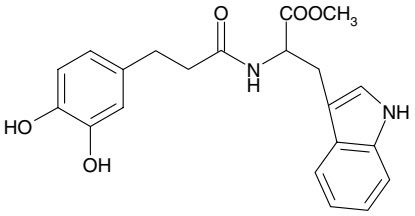


Naringenin 7-*O*-oleic ester **40**



Naringenin 7-*O*-octyl ether **41**

A series of 3,4 dihydroxy hydrocinnamides **42** and **43** exhibited antiatherogenic activity by inhibiting the formation of fatty streaks in high cholesterol fed rabbits.[86]



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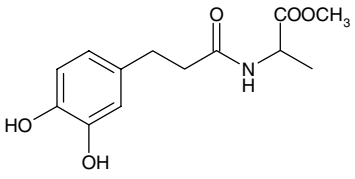

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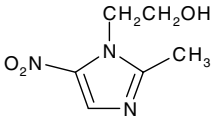


44

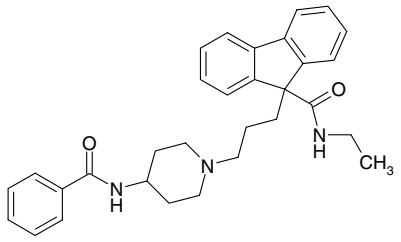
Metronidazole **44** is a member of the imidazole group of compounds that inhibit cytochrome P_{450} enzymes including those involved in cholesterol side-chain conversion, vitamin D synthesis, and progestin and androgen synthesis. These compounds are likely to have direct effect on other lipid fractions as they have been found to increase HDL cholesterol in animals.[87]

7.7. Microsomal triglyceride transfer protein inhibitors

Microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein present in the endoplasmic reticulum of hepatocytes and intestinal cells. A defect in MTP gene (producing a severe deficiency in MTP) caused marked reductions in plasma triglycerides, LDL, and VLDL cholesterol (a beta lipoproteinemia). These findings suggest that synthetic inhibitors of MTP are capable of producing a partial deficiency in MTP function which might be therapeutically useful for inhibiting the production of VLDL and chylomicrons, thereby reducing the levels of atherogenic lipoproteins.[88]

It has also been shown in vitro, that MTP catalyzes the transport of lipid molecules between phospholipid membranes, suggesting that MTP is involved in the synthesis of nascent lipoprotein particles within the lumen and endoplasmic reticulum.[89] MTP catalyzed lipid transport suggests that interruption of the assembly of apo B containing lipoproteins by inhibition of MTP could lead to a reduction in circulating atherogenic lipid levels.[90]

A series of MTP inhibitors **45** has normalized plasma lipoprotein levels in Watanable heritable hyperlipidemic rabbits.[91]



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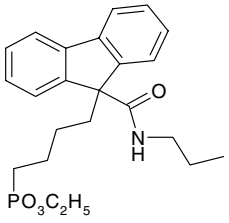


Bristol-Myers Squibb[92] has designed and tested small molecules capable of inhibiting MTP, that can produce marked reductions in atherogenic lipoproteins in hamsters and Watanabe heritable hyperlipidemic rabbits, that lack a functional LDL receptor.

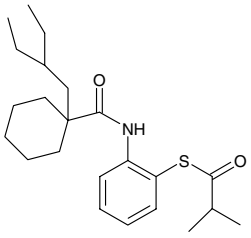
Certain alkyl phosphonates **46** have been synthesized and they also exibit potent MTP inhibition both in vitro and in vivo.[93]

7.8. Cholesteryl ester transfer protein (CETP) inhibitors[94–96]

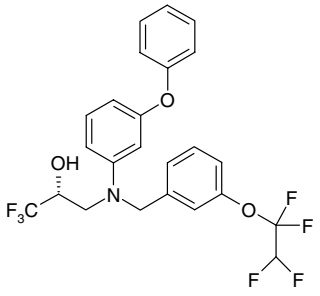
CETP is a hydrophobic glycoprotein that is secreted mainly from the liver and it circulates in plasma, bound mainly to HDL. It promotes the redistribution of cholesteryl esters, triglycerides, and to a lesser extent, phospholipids between plasma lipoproteins. The overall effect of CETP is a net mass transfer of cholesteryl esters from HDLs to TRLs (triglyceride rich lipoproteins) and LDLs, and that of 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 cholesteryl ester transfer protein (CETP), which promotes the transfer of cholesteryl esters from antiatherogenic HDLs to proatherogenic apolipoprotein B (apo B) containing lipoproteins, including VLDLs, VLDL remnants, IDLs, and LDLs. However, there is also evidence that CETP may be involved in reverse cholesterol transport (RCT). Most experimental evidences in animals favor a proatherogenic role for CETP and support a view that inhibition of CETP is antiatherogenic.

It has also been suggested that inhibitors of CETP have the potential to inhibit atherogenesis by enhancing the rate of RCT, 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. JTT-705 **47** is a compound that inhibits CETP activity by form-

ing a disulfide bond with protein. In cholesterol fed rabbits, JTT-705 increased plasma HDL-C, decreased non-HDL-C, and importantly, resulted in a 70% decrease of aortic arch lesions.[97] A trifluoro-3-amino-2-propanol (SC-795) **48** was also reported and this agent inhibits CETP-mediated transfer of [3*H*] cholesterol esters from HDL to LDL in buffer (IC_{50} = 0.02 μM) and in human plasma (IC_{50} = 0.6 μM).[98–100]



JTT-705 **47**



SC-795 **48**

7.9. C-reactive protein (CRP)

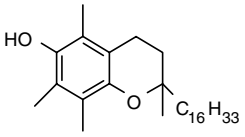
CRP is a member of the pentraxin family which consists of five noncovalently associated peptides surrounding a central core binding of bacterial and fungal polysaccharides. CRP acts as a member of the innate immune system activating the classical pathway of complement fixation and inducing phagocytosis. SAA (serum amyloid A) another acute phase protein is induced by IL-6 (Interleukin) 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 chemoattractant for monocytes as well as mediating lipid delivery to peripheral cells and removal of cholesterol from damaged tissues.[101]

Atherosclerosis is accelerated both directly by effects of cytokines on endothelial cells and indirectly by cytokine effets on the liver, causing increased production of CRP and related factors.[102] CRP induces endothelial cell adhesion molecules and increases endothelial cell monocyte chemoattractant protein-I,[103] inhibits nitric oxide synthesis,[104] increases plasminogen activator (PA-I) expression,[105] and leads to endothelial dysfunction[106] with atherosclerosis prone mice overexpressing CRP showing evidence of increased atherosclerosis. Similarly, there is evidence that SAA may be a mediator of atherosclerosis.[107] 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'.[108]

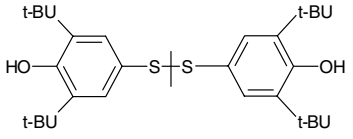
7.10. Antioxidants

7.10.1. Lipid oxidation in atherogenesis.[109] The major target for plasma cholesterol oxidation is suggested to be intimal low-density lipoprotein.[110] Oxidized LDL stimulates foam cell lipid accumulation, as well as, the expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells. This, directly chemotactic for monocytes, increases the expression of monocyte chemotactic protein-1 (MCP-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 the direct effects of oxysterols on the cholesterol efflux machinery[111] as well as the accumulation of undegradable cholesteryl esters in lysosomes. This could 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 LDLs cause injury, apoptosis, and necrosis of vascular cells, which 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, and 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 component of lesion development.

Many antioxidants have been developed to exibit the antiatherogenic activities by inhibiting the foam cell formation in animal model.[112] Vitamin E **49** and probucol **50** as antioxidants have an untoward effect, lowering serum HDL-cholesterol levels. Better results were seen by the introduction of a pyrazoline moiety in place of the disulfide linkage of probucol between the two phenyl rings.[113]

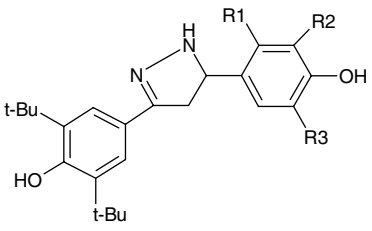


Vitamin E **49**



Probucol **50**

It has been demonstrated that 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-5-(substituted-4-hydroxyphenyl)-2-pyrazolines **51** are active inhibitors of LDL oxidation. These findings need further study to clarify the mechanism of antioxidant action of the pyrazolines in LDL system.[114]



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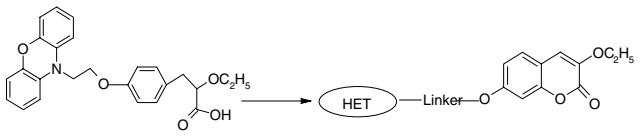
Coumarins are known to have antioxidant potential like tocopherol (Vitamin-E). Previously, Lohray et al.[115] had reported that the 2-ethoxy-3-propanoic acid derivative showed blood glucose lowering activity in experimental animal models. The pharmacophore was changed by cyclizing the 2-ethoxypropanoic acid to from a

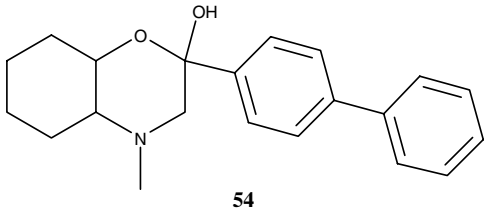
coumarin moiety. Coumarin derivatives **52**, having different heterocycles, attached to them with a linker of one or two carbon chain, show interesting triglyceride lowering activity. Of all the compounds, the ethoxycoumarin derivative with phthalazinone, **53**, was the most potent, showing good triglyceride lowering activity in the swiss albino mouse model.[116]



52 **53**

Conversion of 2-ethoxy propanoic acid to coumarin derivative

2-Biphenylmorpholine derivatives **54**, which are structurally similar to some substituted morpholines, inhibit the ferrous/ascorbate induced lipid peroxidation of microsomal membrane lipids with an IC_{50} value of 250 μM, indicating themselves as potentially antiatherogenic factors.[117]



54

7.11. DGAT (acyl coenzyme A: diacyl glycerol acyl transferase)[118,119]

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. DGAT is a microsomal enzyme that joins Acyl CoA to 1,2-diacylglycerol and as such constitutes the final step in triglyceride biosynthesis. Two distinct forms of DGAT (DGAT-1 and 2) have thus far been identified.

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.[118] The recently cloned DGAT gene is a plausible candidate for the genetic study of obesity. Ludwig et al. recently identified a T79C single nucleotide polymorphism (SNP) in the DGAT gene associated with increased promoter activity.[119]

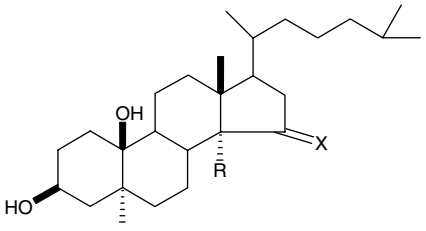
7.12. Lanosterol 14α-demethylase inhibitors

Lanosterol 14α-demethylase is the cytochrome P_{450} monooxygenase, which oxidatively removes the 14α-methyl groups of lanosterol.[120] A number of oxysterols that are suppressors of HMGR (HMG-CoA reductase) activity are catabolites of cholesterol.[121] However, cells which do not oxygenate cholesterol are still capable of regulating the activity of HMGR.[122] HMGR activity and sterol synthesis in these cells can be inhibited by known oxysterol suppressors of HMGR.[123,124] 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 analogue oxygenated at C-32 and their Δ^7 isomers have been shown to decrease the activity of HMGS (HMG-CoA Synthase) in chinese hamster lung cells.[123]

Azalanstat (RS-21607), 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).[125]

The ethenyl lanosterol **55** is reported to function as an irreversible inhibition of rat liver LDM,[126] while the epoxide **56** is a competitive inhibitor with a k_i of 0.6 μM.[127] Lanosterols bearing substitution of the 15 carbon have been the largest due to their expected resistance to elimination of the 14-methyl group as formic acid. The oxime **57** is a modest inhibitor of LDM [(IC_{50}) = 55 μM], but is an effective oral hypocholesteremic agent in hamsters.[128]

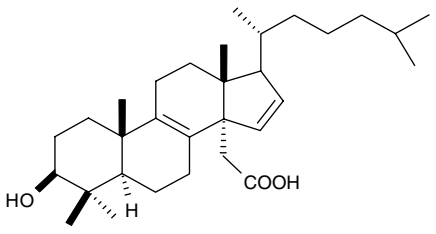


55, R = CHO, Δ^8, X = H_2

56,R =  , Δ^7, X = H_2

57,R = CH_3, Δ^8, X = NOH

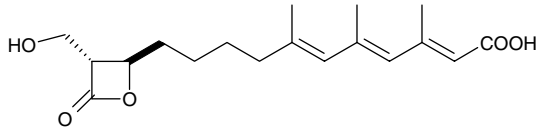
A 32-carboxylic acid derivative of lanosterol (SKF 104976) **58** was found to be a potent inhibitor of lanosterol 14α-demethylase (14 alpha LDM). The 14α LDM activity in a Hep G2 cell extract was inhibited 50% by 2 nM SKF 104976. The rapid inhibition (2–3 h) of HMGR activity by SKF 104976 to 30–60% of the level in controls was not dependent on the initial amount of HMGR enzyme present. These findings suggest that upon inhibition of 14 α LDM by SKF 104976, a mevalonate-derived precursor regulates HMGR activity, even when the sterol synthetic rate is considerably reduced and when HMGR protein levels are very high.[129]



SKF 104976 **58**

7.13. HMG synthase inhibitors

HMG synthase catalyzes the cholesterol biosynthetic step just prior to the reduction of HMG-CoA. Reports have shown L-659, 699 **59** as a potential HMG synthase inhibitor.[130] Tosyl lactams **60** with an IC_{50} value of 2.0 nM were also identified as potent inhibitors of HMG synthase.[131]



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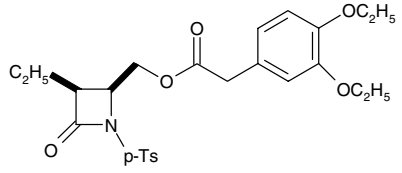
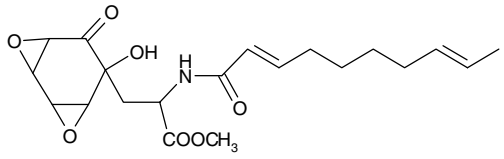


60

A synthetic β-lactone, *trans*-DU-6622 **61**, a mixture of (2*R*, 3*R*) and (2*S*, 3*S*)-beta-lactones, was found to inhibit HMG-CoA synthase (IC_{50}: 0. 15 μM) and pancreatic lipase (IC_{50}: 120 μM). The effects of the optically pure DU-6622 isomers on the two enzymes were compared. The (2*R*, 3*R*)-isomer was shown to be a highly specific inhibitor of HMG-CoA synthase (IC_{50}: 0.098 μM vs 270 μM for pancreatic lipase), while the (2*S*, 3*S*)-isomer markedly increased the specificity of lipase inhibition (IC_{50}: 27 μM vs 31 μM for HMG-CoA synthase). The (2*R*, 3*R*)-beta-lactone is responsible for the specific inhibition of HMG-CoA synthase, while the (2*S*, 3*S*)-beta-lactone is responsible for the inhibition of pancreatic lipase.[132]



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7.14. Squalene synthase inhibitors

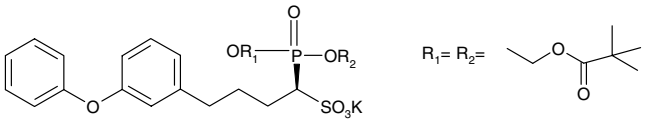
Squalene synthase (SqS) catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate to form squalene.

A potential inhibitor of SS is BMS-188,494 **62** a prodrug, having the ability to lower cholesterol in rats after oral administration, a model insensitive to lipid lowering with statins.[133,134]

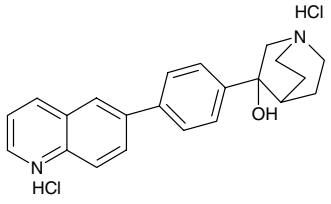
Compound RPR 107393 **63** and its *R* and *S* enantiomers are potent inhibitors of rat liver microsomal squalene synthase, with IC_{50} values of 0.6–0.9 nM. One hour after oral administration to rats, RPR 107393 inhibited de novo [14C]cholesterol biosynthesis from [14C]mevalonate in the liver with an ED_{50} value of 5 mg/kg. The *R* and *S* enantiomers of RPR 107393 (20 mg/kg po qd for 7 days) reduced plasma LDL cholesterol by 50% and 43%, respectively, whereas HDL cholesterol was unchanged. It is an orally effective hypocholesterolemic agent in rats and marmosets and has greater efficacy than lovastatin or pravastatin.[135]

A compound, ER-28448, **64**, a potent and selective inhibitor of squalene synthase, inhibited SqS in rat liver microsomes with an IC_{50} value of 3.6 nM. ER-27856 **65**, another prodrug of although less activity than ER-28448, more potently inhibited cholesterol biosynthesis in rat hepatocytes and also orally inhibited de novo cholesterol biosynthesis in Sprague–Dawley rats, with an ED_{50} value of 1.6 mg/kg.[136,137]

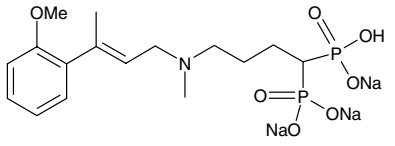
YM-53601, **66**, a squalene synthase inhibitor, lowered not only plasma cholesterol, but also plasma triglyceride levels. It equally inhibited squalene synthase function in hepatic microsomes prepared from several animal species and suppressed cholesterol biosynthesis in rats (ED50 32 mg/kg). In rhesus monkeys, when dosed at 50 mg/kg, twice daily for 21 days, it decreased plasma nonHDL-C by 37%.[138]



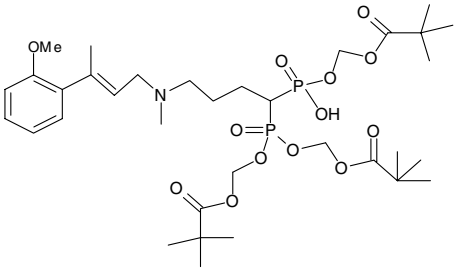
BMS-188,494 62



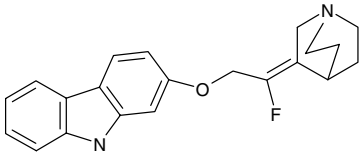
RPR 107393 **63**



ER-28448 **64**



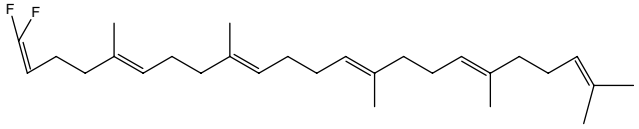
ER-27856 **65**



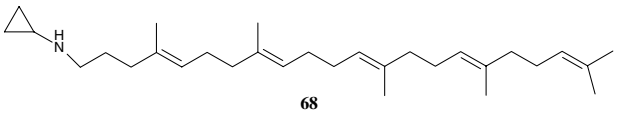
YM-53601 **66**

7.15. Squalene epoxidase inhibitors

Squalene epoxidase catalyzes an important rate limiting step in the biosynthesis of cholesterol, viz squalene epoxide from squalene. The 1,1-difluorosqualene **67** is orally active in mice as indicated by dose dependent reductions in hepatic cholesterol synthesis.[139] Equivalent in vitro potency is seen with cyclopropylamine **68** against rat hepatic SE.[140]



1,1-difluorosqualene **67**



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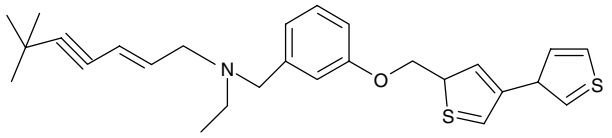
A compound, NB-598 **69**, inhibited the cholesterol synthesis in L6 myoblasts at doses of 1 and 3 μM. Squalene epoxidase inhibitors did not show adverse effects presumably because of the enzyme inhibition downstream of farnesyl synthesis. NB-598 competitively inhibits human squalene epoxidase and strongly inhibits cholesterol synthesis from [14C]acetate in cultured cells. Furthermore, multiple oral administration of NB-598 decreased serum cholesterol levels in dogs.[141]



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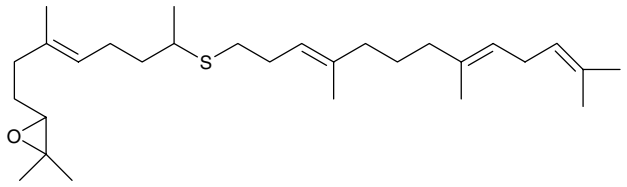
7.16. Squalene 2,3-oxide-lanosterol cyclase inhibitors

Cyclization of 2,3-oxidosqualene (OC) to lanosterol mediated by 2,3-oxidosqualene lanosterol cyclase (OSC) involves the formation of the protosterol cation and its backbone rearrangement to lanosterol. A series of novel sulfur-substituted oxidosqualene (OS) analogues (**70**–**72**) were synthesized and evaluated as OSC inhibitors. In these analogues, C-11, C-15, or C-18 in the OS skeleton were replaced by sulfur. The sulfur position in the OS skeleton was chosen to disrupt one or more key processes involved in cyclization: (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. The analogues were potent inhibitors of mammalian OSCs (IC_{50} = 0.05–2.3 μM for pig and rat liver OSC) and fungal cell-free *Candida albicans* OSC (submicromolar IC_{50} values).[142] The S-18 analogue **72** showed the most potent inhibition toward the rat liver enzyme (IC_{50} = 50 nM) and showed potent, selective inhibition against the fungal enzyme (IC_{50} = 0.22 nM). The $K_i$values ranged from 0.5 to 4.5 μM for pig OSC.



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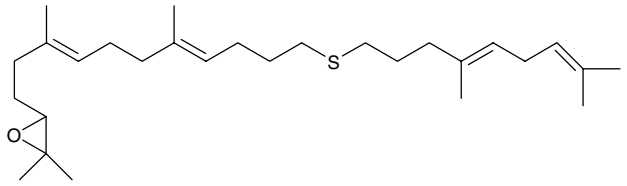

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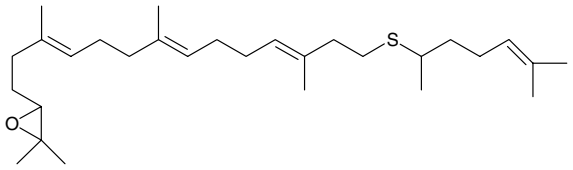

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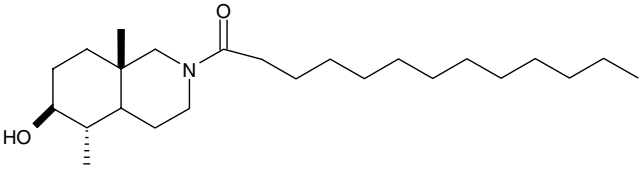
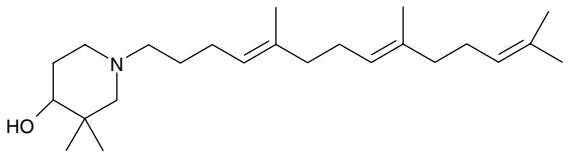


73

Two new azasqualenoid derivatives, bearing a 22,23-epoxy-2-aza-2,3-dihydrosqualene, and its *N*-oxide derivative **73** were studied using rat and pig liver microsomal preparations. Using a solubilized, partially purified squalene 2,3-oxide cyclase, all the compounds exhibited a noncompetitive type of inhibition. Strong inhibition is obtained with an amide such as azadecalin IC_{50} = 0.7 μM.[143] Roughly equivalent activity is observed with the monocyclic analogue **74**.[144] Another new SLC inhibitor, R0 48-8.071 **75**, has shown effective lowering of plasma cholesterol in hamsters and squirrel monkeys when compared to simvastatin.[145]



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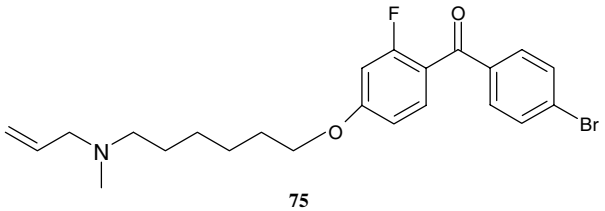
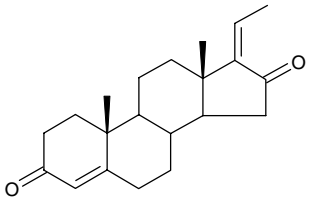


75

7.17. Farnesiod X-receptor antagonists

The bile acid receptor (FXR), a nuclear hormone receptor, when activated by high bile acid level provides a negative feedback loop, that decreases bile acid production by the liver, for example, gugglusterol **76**, which is available as dietary supplement in the US, as gugglu lipid is shown to reduce plasma LDL cholesterol by about 15–18% and triglycerides by about 25–30%.[146]



76

7.18. Sterol regulatory binding protein-cleavage activating protein ligands

Hepatocytes have LDL receptor gene that contains sterol responsive element (SRE), which can be activated by nuclear translocation of an active form of sterol regulatory binding proteins (SREBP-1 and 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 cholesterol levels.

Recently '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, and cholesterol in the blood.[147,148]

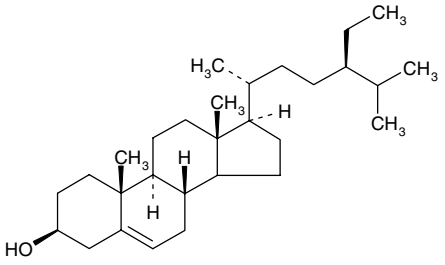
7.19. Synthetic apo E-related peptide

Datta et al.[149] have synthesized a dual-domain peptide, AchE18A-NH(2), 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 h and by 30% at 24 h. 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 investigation of this peptide will be necessary to determine its safety and efficacy.

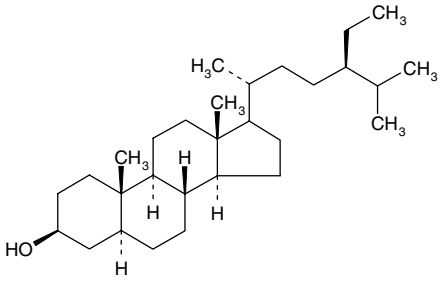
8. Miscellaneous

8.1. Plant sterols[150]

Nonabsorbable cholesterol analogues from plants, due to their structural similarity with cholesterol, inhibit intestinal absorption of cholesterol, for example, β-Sitosterol **77** and Sitostanol **78**.



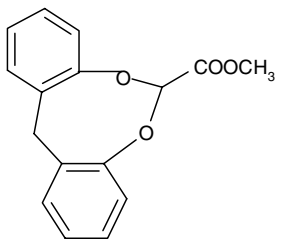
Sitosterol **77**



Sitostanol **78**

8.2. Dibenzodioxocines

Treloxinate **79** is the most important representative of this class possessing blood lipid and cholesterol lowering activity.[151]



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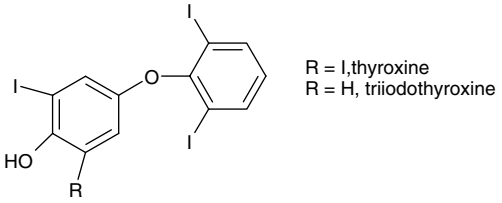


80

8.3. Thyroactive agents

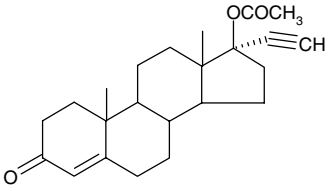
Thyroid hormones, thyroxine and triiodothyroxine **80**, possess activity of reducing serum cholesterol.[152]

8.4. Hormones (e.g., Norethindrone 81)

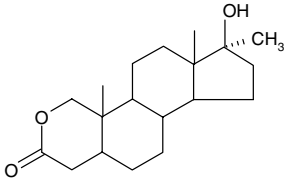
Used mainly in females to decrease triglyceride, VLDL, and chylomicron levels.[153]

8.5. Anabolic steroids (e.g., Oxandrolone 82)

Anabolic steroids reduce hepatic triglyceride levels.[154]



Norethindrone **81**



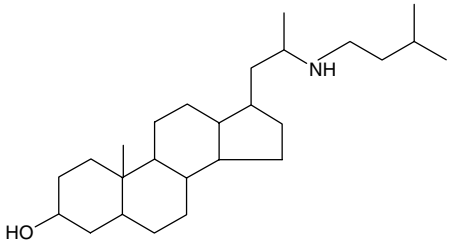
Oxandrolone **82**

8.6. Cholesteromimetic agents (83)

They inhibit β-hydro-β-methylglutaryl CoA reductases, thereby inhibiting first step of cholesterol biosynthesis.[155]

8.7. Nonsteroidal inhibitors of desmosterol reductases and 7-dehydrocholesterol reductase

Triparamol **84** inhibits the biosynthesis of cholesterol by blocking the cyclization of squalene. Boxidine **85** hinders the cholesterol synthesis by inhibiting reabsorption of 9-hydrocholesterol from the bile. It reduces triglycerides and phospholipids.[156]



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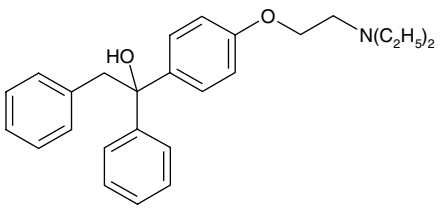
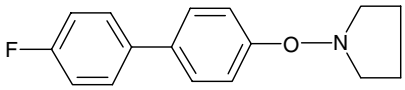
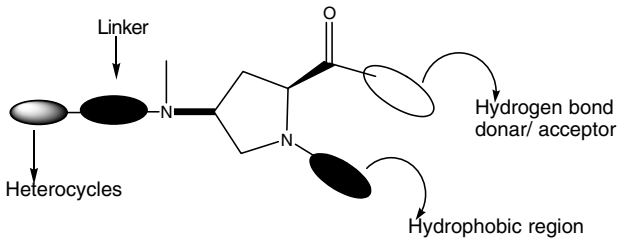


Triparamol **84**



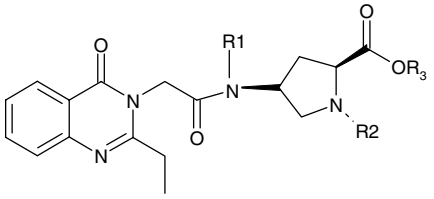
Boxidine **85**

As an ongoing effort to study the specific triglyceride lowering effects, a new scaffold, *cis*-4-amino-L-proline (CAP), as a novel ligand, amenable to three-way functionalization in an orthogonal way was identified. A novel structure in which a heterocycle can be joined to the amino group via a linker and carboxyl group of CAP residue can be kept as such or manipulated to suitable hydrogen donor or acceptor. On the other hand, the ring nitrogen of CAP can be hooked to lipophilic group to strike a balance between lipophilicity and hydrophilicity **86**. Quinazolinone and pyrazolopyrimidone were joined with the amino group of *cis*-4- amino-L-proline through an amide (carboxamidomethyl) linker and thus two novel series of compounds, **87** and **88**, were synthesized.

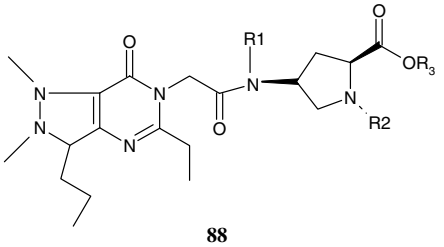


Binding at *cis*-4-amino-L-proline(CAP) **86**

Two compounds exhibit moderate activity toward triglyceride lowering. It is hoped that a little modification could lead to potent triglyceride lowering agents.[157]



87

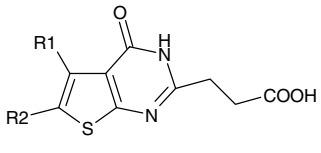


88

8.7.1. 2-Substituted thienopyrimidines as antihyperlipidemic agents. Antihyperlipidemic activity has been reported in some thieno (2,3-*d*)pyrimidine 2-propionic acids,[158] **89**, and some 2-mercaptothieno(2,3-*d*)pyrimidin-4-ones[159] **90**.

A series of 2-substituted thieno(2,3-*d*)pyrimidin-4(3*H*)-ones **91** has been prepared by Shishoo et al.[160] and screened for antihyperlipidemic activity in various animal models. Most of the compounds exhibited good activity. One of the compounds 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno(2,3-*d*)pyrimidin-

4(3*H*)-one **92**(LM 1554, CAS# 89567-03-38) was found to be the most active of all.[161] The serum triglyceride lowering activity exhibited by it has been comparable to clofibrate and riboflavin tetrabutyrate. Compound **92** has been found to be safe during its detail acute and chronic toxicity studies performed on mice and rats.[161]



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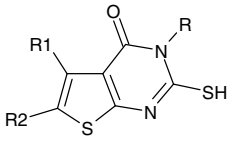

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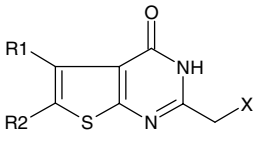
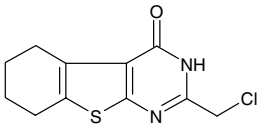


91



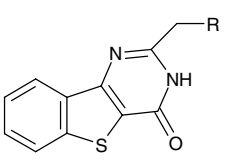
92

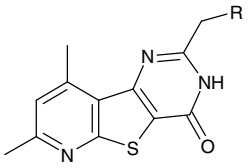
Further, the pharmacokinetics and the mechanism of action of the antihyperlipidemic activity of this compound have been studied in detail.[162] The serum concentrations of this compound have been determined in dogs and rabbits. Poor bioavailability (3–4%) and low volume of distribution of the compound have been observed. This suggests the gastrointestinal tract to be the primary site of action for its antihyperlipidemic activity. This is further substantiated by the observation that the compound is active only orally and not parenterally. The mechanism of action appears to be consisting of inhibition of cholesterol absorption through the gastrointestinal tract.

With the view to complete the SAR studies of the antihyperlipidemic thieno(2,3-*d*)pyrimidin-4(3*H*)-ones two series of isomeric 2-substituted thieno(3,2-*d*)pyrimidin-4(3*H*)-ones **93** and **94** have been synthesized by same workers and evaluated. They have done a detailed QSAR study, which has indicated the direct positive influence of the electronic nature of the 2-substituents of these compounds on their antihyperlipidemic activity.[163]

Jain et al.[164] have recently reported the synthesis and antihyperlipidemic activity of novel condensed 2-chloroalkyl-4-chloro-5,6-disubstitutedpyrimidines **95**. Of these the 4-chloro compounds have exhibited much superior antihyperlipidemic activity compared to all the earlier reported analogues. These compounds significantly reduced serum cholesterol, triglycerides and also elevated the serum HDL levels. The evaluation is by the model, Triton WR 1339 induced hyperlipidemic in albino Wistar rats.

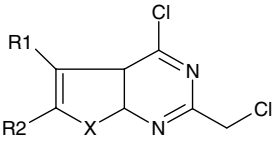
A 3D QSAR study has also been performed to delienate the effects of the 5 and 6 substitutes of these compounds on the antihyperlipidemic activity.





R = H, Cl, OCH_3, OC_2H_5, OH, $OCOC_6H_5$

93 **94**



X = S, HC=CH, etc, R^1, R^2 = H, alkyl, aryl, cycloalkyl, heteroalkyl

95

9. Conclusions

CHD remains a leading cause of death and disability. Prevention through risk factor controls like smoking cessation and control of blood pressure, blood glucose, and LDL cholesterol, and raising of HDL cholesterol remains the most effective long-term option for the treatment. Development of additional cholesterol-lowering agents with mechanisms of action distinct from statins will probably be necessary to achieve cholesterol target levels in many individuals. Several attractive pharmacologic targets have been identified, and agents that act on those targets, when used alone or in conjunction, should be very effective cholesterol-lowering therapies.

In spite of extensive research and development of numerous drugs, antihyperlipidemic therapy is still deprived of the efficiency, safety, thorough knowledge of the exact mechanisms of the real cause of hyperlipidemia, and finally 'cost'.

Following problems still need to be solved in the drug therapy of Hyperlipidemia.

- New drugs are required to cover the hitherto untreatable cases of Type II hyperlipidemia, wherein drugs like clofibrate, nicotinic acid, *d*-thyroxin, etc. are used without much success.
- Also new drugs are needed to be discovered, that will be able to block the stimuli causing the formation of an atherosclerotic lesion.
- Furthermore, drugs are needed to be developed, to bring about regression of the already existing atherosclerotic lesions.
- The most widely used 'statins' also suffer from limitations like, intolerance and adverse effects, ineffectiveness or only partial effectiveness in lowering of cholesterol levels only upto maximum 40% risk reduction, and finally the 'cost'.

Thus, there is still need for development of better antihyperlipidaemic agents. Medicinal chemists all around

the world have been designing, synthesizing, and evaluating a variety of new molecules for antihyperlipidaemic activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.04.031.

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14. http://www.lipidsonline.com. (This site has been designed and developed by CCIT—The Center for Collaborative and Interactive Tehnologies at Baylor college of medicine in Houston, Texas for educational resources in atherosclerosis).
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