Summary

and

Conclusion

Chapter 1

CHD remains a leading cause of death and disability. Prevention through risk factor like smoking cessation, control of blood pressure, blood glucose, and LDL cholesterol and raising of HDL cholesterol remains the most effective long-term option for 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. Drugs with different targets have been discussed in this chapter, when used alone or in conjunction, can be very effective cholesterol-lowering therapies. A careful study of literature indicated ezetimibe as most widely used drug but with some drawbacks and hence it was envisaged to develop its analogs bearing azetidin-2-one moiety.

Chapter 2

β-lactam i.e., azetidin-2-one is a versatile biologically important scaffold, as well as, synthon for various biologically important compounds thus, making it an appealing target for medicinal chemists all over the world. An attempt has been made, in this chapter, to review majority of the reports on the biological and pharmacological profiles of azetidin-2-one derivatives alongwith an overview on their chemistry and synthesis.

Chapter 3

In all 20 novel condensed 2-(1-(substitutedphenyl)-4-oxo-azetidin-2-yl)pyrimidin-4(3*H*)-ones (**V*i*–*xx***) were synthesized and screened in Triton WR 1339-induced hyperlipidemic rats, for antihyperlipidemic activity and some of these compounds were found to be comparable to the standard drugs, gemfibrozil and ezetimibe. Compound **V*iii*** was found to be the most active of all the compounds, exhibiting significant lipid-lowering effects like reducing serum levels of cholesterol and triglycerides in test animals. Other derivatives **V*viii***, **V*iii*** and **V*i***, also significantly elevated the serum HDL levels in test animals. Hence, this series could be further structurally modified and evaluated to obtain potential antihyperlipidemic agents which can help in lead optimization.

Chapter 4

In all 24 analogs of ezetimibe were designed (**VIII*i*–*xvi***) and (**X*i*–*viii***) and prepared on the basis of a docking study at NPC1L1. The synthesized 2-azetidinone derivatives

were characterized and evaluated in Triton WR 1339 induced hyperlipidemic rats, for antihyperlipidemic activity. The investigation showed compounds **VIII*iii*** and **X*iii*** exhibited significant antihyperlipidemic activity, which is comparable to the standard drug, ezetimibe. A certain degree of correlation between the docking scores and antihyperlipaemic activity of the compounds *vis-a-vis* the standard drug was also observed.

Chapter-5

Current new drug discovery efforts to develop new molecules for antihyperlipidemic research involve focussing on various new molecular mechanisms of hyperlipidemia and thereby several attractive molecular targets involved thereof in this process are being exploited. This chapter discussed twenty such molecular targets that could offer an insight for developing new leads for antihyperlipidemic therapy to researchers.

NPC1L1 protein plays critical role in cholesterol absorption. Blocking NPC1L1 endocytosis can dramatically decrease the cholesterol internalization and formation of atherosclerosis. PPAR agonists mostly exert their anti-atherosclerotic properties by multiple mechanisms. Activation of PPAR-α, PPAR-δ and PPAR-γ can be promising in treatment of atherosclerosis. Of these, PPAR-δ is a better target, while PPAR-α and PPAR-γ stimulations play complementary roles in the prevention of atherosclerosis.

ACAT catalyzes the formation of cholesteryl esters from cholesterol. The inhibition of ACAT activity has been associated with decreased plasma cholesterol levels by suppressing cholesterol absorption. CoQ10 is known to diminish the LDL cholesterol settings of oxidative stress. Thus, CoQ10 is beneficial in treating and preventing atherosclerosis.

Inhibition of ACL reduces plasma LDL cholesterol by inhibiting cholesterol synthesis & decreases plasma triglyceride levels by reducing fatty acid synthesis.High concentration of HDL results in antiatherogenic property by clearing cholesterol from cells and delivering this free cholesterol to liver. HDL promotes and facilitates the process of reverse cholesterol transport and thus, protects against the progression of atherosclerosis. Therefore HDL enhancement is an important stratergy for treatment of atherosclerosis.

Drugs that inhibit MTP prevent the assembly of apo B-containing lipoproteins thus, inhibiting the synthesis of chylomicrons and VLDL and leading to decrease in LDLC. Inhibition of CETP can lead elevate HDL levels and atherosclerosis inhibition.

Similarly inhibition of CRP is a good strategy to control atherosclerosis and hyperlipidemia. Lipid oxidation inhibition is one of the important contributors of atherogenesis. Many antioxidants have been developed to exhibit the anti-atherogenic activities by inhibiting lipid oxidation.

DGAT catalyzes the final step in the triglyceride synthesis. Its inhibition can be atheroprotective. LDM is the cytochrome P450monooxygenase involved in cholesterol synthesis. Its inhibition is another strategy for the control of hypercholesterolemia.

HMG synthase catalyzes an important cholesterol biosynthetic step and thus, its inhibition is also one of the strategies for antihyperlipidemic therapy. SqS catalyses the reductive dimerization of two molecules of farnesyl pyrophosphate to form squalene. Inhibition of this enzyme has also been demonstrated to upregulate LDL receptor activity, thus inhibition of SqS can be a good therapeutic strategy for lipid lowering.

SQLE represents one of the key and rate-limiting enzymes of the mevalonic acid (MVA) pathway. For this reason, SQLE plays a pivotal role in cholesterol homeostasis maintenance and can be inhibited to control hyperlipidemia. OSC offers itself as an attractive target for inhibition by novel ligands for antihyperlipidemic therapy as it plays an important role in lipid biosynthesis.

FXR is mainly expressed in the liver, intestine and kidney and it plays an essential role in bile acid/cholesterol homeostasis. Activation of FXR increases reverse cholesterol transport *via* scavenger receptor class B type I (SR-BI) -dependent and SR-BI-independent pathways and can be useful therapeutic strategy.

SREBPs (SREBP-1 & SREBP-2) regulate transcription of LDL receptor & genes required for cholesterol, fatty acid, triglycerides & phospholipid synthesis and therefore Inhibition of SREBPs decreases the biosynthesis of cholesterol and fatty acid, making it a useful approach.

ApoE plays an important role in lipid metabolism through regulating the uptake of lipoproteins from the circulation by receptor-mediated endocytosis. Its inhibition results in greatly increased susceptibility to the development of atherosclerosis. Therefore, stimulation of ApoE has therapeutic significance. PCSK9 is a secreted protein that influences plasma levels of low-density lipoprotein cholesterol (LDL-C) and susceptibility to coronary heart disease. It plays a major regulatory role in cholesterol homeostasis. Blocking the interaction between LDLRs and PCSK9 is likely to reduce

CV risk in people by increasing the availability of cell-surface LDLRs and reducing serum LDL-C.

Chapter-6

LM-1554 (2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one; CAS #89587-03-3), and LM-1576 (4-Chloro-2-chloromethyl-5-(4-chlorophenyl)-thieno[2,3-*d*]pyrimidine) have shown promising antihyperlipidemic activity in preclinical evaluation and also found to be safe in their toxicity studies warranted an investigation into their pharmacodynamics. The technique of molecular docking was utilised for analysing the orientation of conformations and poses, as well as, assessing favourability of interactions of LM-1554 and LM-1576 into the binding pockets of six different molecular targets related to hyperlipidemia. This was done to gain some insights in their probable mechanism of action, as an antihyperlipidemic entity. The compounds seemed to be acting through the inhibition of NPC1L1. ACL also could be their molecular target to some extent. Thus, on this basis, selective *in-vitro* assays involving these two targets could now be the next step to confirm its mechanism of action.

A new one-pot synthetic protocol for synthesis of LM-1576, has been developed under MWI using dual use of $POCl_3$ as a chlorinating agent, and acid catalyst for the cyclocondensation of the *o*-aminoester substrate. This protocol was found to be more efficient and less time consuming than the conventional one.

In-vivo and *in-silico* studies suggested LM-1576 as more promising antihyperlipidemic entity than LM-1554. Hence, a simple, sensitive and reliable method for the determination of LM-1576 over the concentration range of 25–6400 ng/ml in rabbit serum by HPLC was developed and validated. The method consisted of sample preparation by protein precipitation and extraction into mobile phase, followed by chromatographic separation and UV detection. No interfering peaks were observed at the elution times of LM-1576 and I.S. The method was accurate, reproducible, specific and applicable to the evaluation of pharmacokinetic profiles of LM-1576 in rabbits. The developed HPLC method was found to be suitable for the analysis of LM-1576 in rabbit serum and used for the estimation of pharmacokinetics parameters after administration of LM-1576 by oral route, where it showed good rate of absorption, shorter half-life and higher rate of elimination.