Inhibitors of HMG-CoA Reductase: Current and Future Prospects

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Abstract: High levels of cholesterol are a primary risk factor in the development of cardiovascular diseases. In this review, we have summarized the structural, chemical and computational aspects of hypocholesterolemic drugs, both statins and non-statins, that target enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) to block cholesterol biosynthesis.

Key Words: 3-hydroxy-3-methylglutaryl-coenzyme A reductase, α-asarone, cardiovascular disease, low density lipoproteins, cholesterol, statins, side effects, structure-based drug design.

INTRODUCTION

It is well documented that cardiovascular disease (CVD) accounts for more deaths than any other disease. According to World Health Organization (WHO) estimates in 2005 17.5 million people died of CVD, which is 30 percent of all deaths globally [1]. The 2005 mortality rate data also show that nearly 2400 Americans die of CVD each day (an average of 1 death/37 sec) [2]. Today, in most of the developed and developing countries, dyslipidemia (and subsequently atherosclerosis) is the leading cause of CVD related illness and deaths [3, 4]. The American Heart Association estimates that 34.5 million adults in the US have high cholesterol, and an additional 100 million are thought to have levels considered borderline to high [5]. Risk factors include tobacco smoke, high blood cholesterol, high blood pressure, physical inactivity, obesity and overweight, and diabetes mellitus [1].

Cholesterol is a byproduct of the mevalonate pathway. The name of this pathway originates from the carboxylic acid mevalonate, the precursor of all isoprenoids and sterols in the organisms, which biosynthetically precede from enzymatic reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) [6, 7]. Despite acquiring bad reputation, cholesterol is required in mammals and is considered crucial for normal cellular function. In fact, cholesterol is a major structural component of cell membranes, a precursor for the synthesis of bile acids, and a participant in repairing damaged tissues. It is a substrate for the synthesis of steroid hormones, a precursor for vitamin D, and an integral factor in embryonic morphogenesis, signal transduction, and sperm development. However, the over accumulation of cholesterol is toxic and well known to play a central role in CVD [8]. Noteworthy is the fact that cholesterol is not an essential requirement of daily food intake as humans are capable of synthesizing it [9, 10]. Its biosynthesis in the body is mainly regulated in the liver by the enzyme HMG-CoA reductase (HMGR) [7]. Inhibition of this enzyme has proven to be one of the most effective approaches for lowering low density lipoproteins (LDL-C), and eventually reducing CVD [11]. Mechanistically, the inhibition of HMGR leads to the increase production of LDL receptors by activating the sterol response element-binding protein (SREBP), a transcription factor that promotes expression of the LDL-receptor gene, [12] which reduces LDL-C levels in systemic circulation [13].

Currently, approximately 12 million Americans take cholesterol-lowering drugs, including of at least 14 brands in seven major drug classes, with sales surpassing $24 billion in 2007 [14]. Among them, the HMGR inhibitors (known as statins) represent the highest standard in treating hypercholesterolemia, and few other drugs are prescribed as often because they do not reach the same tolerance and efficacy [15, 16].

The focus of this review is to cover the latest advances in statins and other HMGR inhibitors in terms of their structure-mechanism relationship, classification, side-effects, and optimization of their selectivity. Detailed structural characteristics of HMGR, computational studies, and future perspectives will also be discussed.

1. STRUCTURE OF HMG-CoA REDUCTASE

HMGR is a rate-limiting and polytopic transmembrane glycoprotein that catalyzes a key step in the mevalonate pathway, which is involved in the synthesis of essential natural compounds including sterols such as cholesterol, heme, ubiquinones, dolichols, farnesylated and geranylgeranylated proteins, isoprenoid-derived hormones, and vitamin D [6, 17]. In contrast to other late-stage intermediates in this pathway, 3-hydroxy-3-methylglutaric acid (HMG) is water soluble and, if HMGR is inhibited, there are alternative metabolic pathways for its breakdown, so that there is no build-up of potentially toxic precursors. This makes HMGR an attrac-

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tive target for contemporary cholesterol-lowering drugs [18]. In fact, as mentioned above, inhibition of HMGR has proven to be one of the most efficient therapies to lowering LDL-C.

The human *hmgr* gene is located on chromosome 5 and is over 24.8 kb long encoding three domains: the membrane-anchor domain from 10-339 residues, a flexible linker region from 340-459 residues and the catalytic domain from 460-888 residues, resulting in a polypeptide 888 residues long [19]. The monomer of the catalytic domain is further divided into three domains: an N-terminal 'N-domain' from 460–527 residues, two large 'L-domains' from 528–590 and 694–872 residues, and a small 'S-domain' from 591–682 residues. The comparison of amino-acid sequences and phylogenetic analysis of over 150 known HMGR sequences has revealed two major classes of HMGR: The Class I enzymes of eukaryotes and some archaea bacterias; and the Class II enzymes of certain eubacteria and most of the archaea bacterias [20, 21]. Unlike the poorly conserved membrane-anchor domain, the catalytic domain is highly conserved in eukaryotes. High resolution crystal structures of both HMGR classes, Class I for humans HMGRh [22-28] and Class II from the soil bacterium *Pseudomonas mevalonii* HMGRp, [29, 30] have been determined in complexes with either the natural substrate (e.g., HMG-CoA), or the coenzyme (NADPH or NADH), or both, or known inhibitors of the statin type. The crystal structures have provided mechanistic details on HMGR function and inhibition by substrate and statins, respectively. Information of all the currently available 27 HMGR crystal structures from both classes with their Protein Data Bank (PDB) code, co-crystallized ligands, and references are summarized in Table **1**.

Table 1. Current known HMGR Crystal Structures in the Protein Data Bank

PDB	Co-Crystallized Ligand	Reference
1DQ8	HMG	[23]
	CoA	
1DQ9	HMG-CoA	
1DQA	NADP	
	HMG	
	CoA	
1HW8	Compactin	[22]
	ADP	
1HW9	Simvastatin	
	ADP	
1HWI	Fluvastatin	
	ADP	
1HWJ	Cerivastatin	
	ADP	
1HWK	Atorvastatin	
	ADP	
1HWL	Rosuvastatin	
	ADP	
2Q1L	Modified atorvastatin	[27]
2Q6B	Modified atorvastatin	[26]
2Q6C	Modified atorvastatin	
2R4F	Pyrazol based	[25]
3BGL	Pyrrol based	[24]
3CCT	Pyrrol based	[28]
3CCW	Imidazol based	
3CCZ	Imidazol based	
3CD0	Imidazol based	
3CD5	Pyrrol based	
3CD7	Pyrrol based	
3CDA	Pyrrol based	
3CDB	Pyrrol based	
1QAX*	NAD	[29]
	HMG-CoA	
1QAY*	Mevalonate	
	NAD	
1R31*	Mevalonate	To be published
	CoA	To be published
1R7I*	---	To be published
1T02*	Lovastatin	[30]

(Table 1. Contd….)

* From Pseudomonas mevalonii.

The structural comparison of both HMGR classes also shows that HMGRh is a tetramer of identical subunits and has all the three domains; meanwhile, HMGRp only has the catalytic domain, and it is a trimer of identical dimers. The dimeric active site of both classes that binds HMG-CoA is formed by residues from each monomer along with a non-Rossmann type coenzyme-binding site in humans or in bacteria. The active site residues that participate in the catalytic reaction are also well conserved in both classes. This means that HMGRp is also a potential drug target for antibacterial compounds [31]. Compared to these similarities, the major differences are in the N- and C-terminal parts of the catalytic domains. In HMGRh, the catalytic residue Lys691 is found on the monomer that binds the HMG-CoA and comes from the associated loop, called *cis-loop*, that folds over part of the HMG-binding pocket. The *cis-loop* is called so because it contains a *cis*-peptide between residues C688 and T689. In HMGRp, the *cis-loop* is not present and the catalytic residue Lys267 comes from the monomer subunit that binds NADPH.

Although statins, which mimic in part the HMG moiety substrate in HMG-CoA, competitively bind in the same pose as the substrate in both classes, the specific interactions with the binding site residues are significantly different. A major difference is in the interaction of the hydrophobic portion of statins with the *cis-loop* in HMGRh that is absent in HMGRp. This may explain, at least in part, the ~100 fold K_i difference in inhibition of HMGRh by statins as compared to HMGRp.

The HMGRh is located on the endoplasmic reticulum (cytoplasm for HMGRp) and catalyzes the four-electron reductive deacylation of HMG-CoA to CoA and mevalonate as follows: [23]

(*S*)-HMG-CoA + 2NADPH + $2H^+ \rightarrow$ (*R*)-mevalonate + $2NADP^+$ + CoASH

Some of the key amino acid residues and interactions of HMGR that bind to the HMG-CoA substrate include (Fig. (**1**); Ref. [23, 29]):

In HMGRh, Lys735 makes electrostatic interactions with the C5 anionic carboxylate group of HMG, which in turn holds the substrate to the enzyme. Ser684 also makes an H-bond with this group and further stabilize the substrate. In HMGRp, only Arg261 makes a single H-bond with the C5 anionic carboxylate group.

- The C3-OH group of the substrate is also stabilized by two residues, Arg590 and Asp690. Both make an H-bond with the hydroxyl hydrogen.
- In HMGRh, Lys691, a part of the *cis-loop*, makes electrostatic interactions with the carbonyl oxygen of C1, which is reduced to a primary alcohol. The C1 carbonyl oxygen is also in the H-bonding distance of Glu559 and Asn755 which helps in the proper orientation of the substrate for reduction. In HMGRp, only Lys267 makes an H-bond with the C1 carbonyl oxygen group.
- In HMGRh, Tyr479 makes hydrophobic interactions with the adenine base of the CoA portion of the substrate, and clamps down the binding pocket for effective reduction by the cofactor, whereas Ala751 and Leu853 makes hydrophobic contacts with the HMG part. In HMGRp, Ala368, Ile713, and Leu372 make hydrophobic contacts with the HMG part.
- In HMGRh, His866 and Glu559 act as a proton donor (Glu-559 and Asp-767 help to elevate the pK_a of the carboxylate, so that a higher proportion is protonated at physiological pH) to the sulfur atom of the thioester (SCoA) that is reduced to CoASH. The mevaldehyde intermediate is further metabolized into many important sterol and nonsterol products [7] like farnesol, farnesyl pyrophosphate, and cholesterol [32-34].

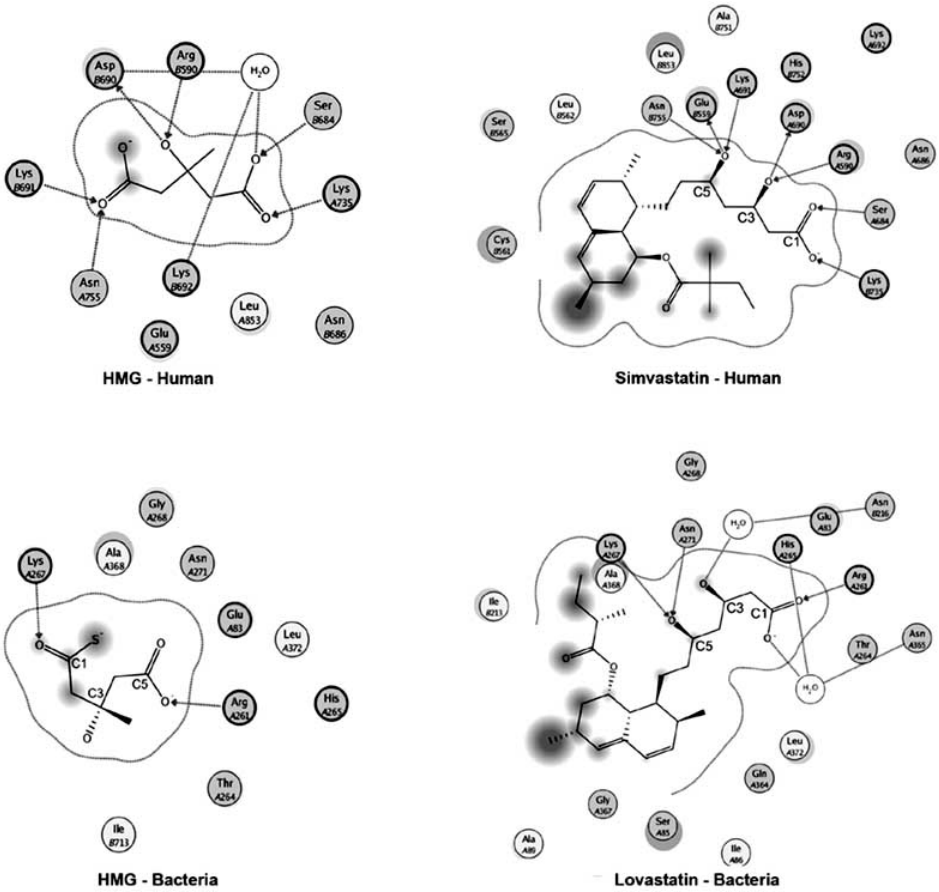


Fig. (1). Comparison of the substrate and statin-binding sites from both human and bacterial enzyme HMGR. The residues are numbered according to the numbering in crystal structures of PDB code 1DQ8 (HMG-Human), 1QAX (HMG-Bacteria), 1HW9 (Simvastatin-Human), and 1T02 (Lovastatin-Bacteria). The figure legend is explained in Fig (**3**).

2. HMG-CoA REDUCTASE INHIBITORS

2.1. History and Classification

Before the discovery of HMGR inhibitors, the lipid-lowering prescriptions were largely limited to suggestions of dietary changes and medications such as bile-acid sequestrants (cholestyramine and colestipol), nicotinic acid (niacin), fibrates (such as gemfibrozil, fenofibrate, bezafibrate and others) and probucol. The limited efficacy or tolerability of these medications prompted scientists to look for alternatives. The first natural inhibitory product for HMGR was discovered in the mid-1970s by Endo and coworkers in a fermentation broth of *Penicillium citrinum*, later called compactin [35-38]. In the late 1970s, Merck Research Laboratories found another potent inhibitor, mevinolin (later called lovastatin) in a fermentation broth of *Aspergillus terreus* [39]. In 1988, the slightly modified form of lovastatin (with an additional methyl group), called simvastatin, [40] was the second approved statin drug. After that, three more so called first-generation statins (which show a modest ability to lower lipids in humans with an LDL-C reduction of 20–40%) and three second-generation 'super-statins' (that elicit greater reductions of 40-60% in LDL-C) were approved within a span of 15 years: pravastatin (discovered by Sankyo after the failure of compactin) in 1991, [41] fluvastatin in 1994, [42, 43] atorvastatin (super-statin) in 1997, [44] cerivastatin (a super-statin that was later discontinued) in 1998 [45] and the latest rosuvastatin (super-statin) in 2003 [46]. Currently, statins represent the mainstay of cholesterol lowering treatment and various studies like 4S, [47] CARE, [48] LIPID, [49] WOSCOP, [50] AFCAPS/TexCAPS, [51] REVERSAL [52] and the latest JUPITER [53] have provided ample evidence supporting the use of statins in dyslipidemia for primary and secondary prevention of CVD.

While all these statins share a common mode of action, they differ in their overall structural, biochemical, thermodynamic and pharmacokinetic properties, which markedly affect their overall efficacy, safety, and other non-LDL actions. All these properties are systematically detailed in Table **2**.

As mentioned previously, all statins share the same HMG-like moiety, but differ in the hydrophobic backbone attached to this moiety. Statins are classified into two types [22]. Type I statins (lovastatin, pravastatin and simvastatin) are natural fungal products that are administered as lactone pro-drugs (pravastatin is an exception). The lactone ring is hydrolyzed in-vivo by various cellular enzymes (esterases) to generate the carboxylic acid [39]. In contrast, type II statins are fully synthetic and are administered as salts of carboxylic acids. Structurally, type II statins are characterized by the presence of larger hydrophobic regions than those in type I. One of the main distinguishing features of the type II statins is the attached fluoro-phenyl groups [18, 31, 63].

2.2. Interactions with HMGR

Crystallographic structures of statins and HMG-CoA bound to HMGR [57] reveal that the carboxylic acid of statins mimics the carboxylic acid of HMG-CoA. Fig. (**2**) shows the superimposed statins and the common binding site residues of HMGRp involved from 11 co-crystallized structures. For reference, the substrate binding site is also shown. It is noteworthy that the HMG-moiety of all the statins shows a high degree of overlap compared to the HMG substrate and involves the same set of binding site residues.

Statins exist as anions at pH 7.4, which is critical to their ability to compete for the HMGR active site by anchoring *via* an electrostatic bond to cationic Lys735. HMGR is stereoselective and preferentially binds to the (3*R*,5*R*) isomer of the statins. Similar to the binding interactions of HMG-CoA, the HMG-like moiety of the statins makes H-bonds with residues Glu559, Lys735, and Asn755 from one monomer, and with Arg590, Ser684, Asp690, and Lys691 from a second monomer, as shown in Figs. (**2**) and (**3**). All these interactions play an important role in the tight binding of the statins which act as competitive inhibitors and resemble the interactions of the natural substrate. The hydrophobic part of the statins is composed of various hydrophobic ring systems that yield different relative affinities with the enzyme (Table **2**).

Specific interactions involved in statin-HMGR binding (similar to those for substrate binding) are described as follows (see also Figs. (**1**-**3**); Ref. [22-28]):

- Lys735 makes electrostatic interactions with the anionic C1 carboxylate oxygen atom of simvastatin, atorvastatin, fluvastatin, and rosuvastatin. Ser684 also makes an H-bond with the other oxygen of C1 carboxylate. In HMGRp, Arg261 is equivalent to Lys735 in making this interaction as observed in the crystal structure of bound lovastatin in PDB code 1T02 (Fig. (**2**)).
- Lys692 also makes an ion-dipole interaction with this second oxygen of C1 carboxylate of statins (Fig. (**2**). In HMGRp, Thr264 is equivalent to Lys692 in making this interaction).
- Asp690 and Arg590 interact in an ion-dipole bond with the hydrogen of the C3-OH group of statins.
- Glu559, Asp767 and Lys691 make H-bonds with the C5-OH of statins. In HMGRp, Glu83, Asn271 and Lys267 are equivalent to human Glu559, Asp767 and Lys691, respectively, for interaction with the C5-OH group.

Interactions dissimilar to those for substrate binding are the following:

- Ser661 makes an H-bond with the p-fluorophenyl group of atorvastatin (and inhibitors of co-crystallized structures in PDB codes 2Q6B, 3BGL, 2R4F and 2Q1L; (Table **1**)). An additional bond at this location is also observed with Arg590 in the crystal structure 2Q1L (Fig. (**3**)).
- Atorvastatin and especially rosuvastatin exhibit the strongest binding interactions. These two statins are also the only ones which establish a H-bond between their hydrophobic region and the two serine residues of the enzyme. The hydroxyl group of Ser565 makes an H-bond with the carbonyl (C18) oxygen of atorvastatin, while one of the oxygen atoms of the sulfonyl group in rosu-

Table 2. Selected Biochemical, Structural, Thermodynamic and Pharmacokinetic Parameters of Statins

Property		Atorvastatin	Fluvastatin	Pravastatin	Rosuvastatin	Simvastatin	Lovastatin
Profile	Source (synthetic/natural)	Synthetic Derived from pyrrole	Synthetic Derived from indole	*P. citrinum* &*Streptomyces* (various stains)	Synthetic	Methylation of lovastatin	*Aspergillus terreus*
	Brand name(s)	Lipitor®	Lescol®	Pravachol®	Crestor®	Zocor®	Mevacor®
	Company	Pfizer	Novartis	BMS	AstraZeneca	Merck	Merck
	Year approved (FDA)	1997	1994	1991	2003	1988	1987
	Patent Expiry	2010/2011	2011	2006	2012	2006	2001
Biochemical	IC_{50} (nM) enzyme	6.2±1.7	28	31.6±4.4	3.1±0.4	4.3±1.3	---
	IC_{50} (nM) hepatocytes	2.5±0.8	---	29±4	0.6±0.1	6.2±1.3	---
	IC_{50} (nM) myocytes	78±22	---	1519±514	65±17	27±0.8	---
	K_i (37 ^{O}C)	14±1	256±33	103±27	2.3±0.4	7±17	0.6
	Rat ED_{50} ₣	0.11	---	0.68	0.35	0.4	---
Structural	X-ray (PDB code)	1HWK	1HWI	---	1HWL	1HW9	---
	Chemical formula	$C_{33}H_{35}FN_2O_5$	$C_{24}H_{26}FNO_4$	$C_{23}H_{36}O_7$	$C_{22}H_{28}FN_3O_6S$	$C_{25}H_{38}O_5$	$C_{24}H_{36}O_5$
	Moiety (ring system)	Pyrrole	Indole	Reduced naphthylene	Pyrimidine	Reduced naphthylene	Reduced naphthylene
	Membrane location (PL=Phospholipid)	PL acyl chains (upper)/glycerol backbone	---	PL headgroup	PL headgroup/glycerol backbone	PL acyl chains (upper)/glycerol backbone	PL acyl chains (upper)/glycerol backbone
	Crystal contacts within 4.2 Å (Hydrophobic/All)	19/91	15/70	---	15/79	11/61	---
	Mol. Wt.	558.6	411.5	424.5	481.5	436.6	422.6
	Rotatable bonds	13	8	11	10	11	11
	ASA (unbound to bound in $Å^2$)	1060	870	---	880	880	---
	HBA	5	4	6	8	5	5
	HBD	5	4	5	4	4	4
	H-bonds	10	8	---	9	8	---
Thermodynamic	Enthalpy (ΔH) †	-4.3±0.1	~0	-2.5±0.1	-9.3±0.1	---	---
	Entropy (-TΔS) †	-6.6±0.6	~-9.0	-7.2±0.4	-3.0±0.7	---	---
	Free energy (ΔG) †	-10.9±0.8	-9.0±0.4	-9.7±0.4	-12.3±0.7	---	---
Pharmacokinetic	Prodrug	No	No	No	No	Yes	Yes
	HPLC Log P	3.76	---	2.2	2.4	4.84	---
	Comparative lipophilicity	Yes (++++)	Yes (++++)	No (+)	No (++)	Yes (+++++)	Yes
	LDL-reduction ‡	42-46	22	23-29	52-55	30-40	24-27
	Log D	1.0 to 1.25	1.0 to 1.25	-0.75 to -1.0	-0.25 to -0.05	1.5 to 1.75	---

(Table 2. Contd….)

Property		Atorvastatin	Fluvastatin	Pravastatin	Rosuvastatin	Simvastatin	Lovastatin
	Metabolism	CYP3A4	CYP2C9	Sulfation	CYP2C9	CYP3A4	CYP3A4
	Bioavailability (%)*	12	19-29	18	20	5	5
	Half-life (hr)	15-30	0.5-2.3	1.3-2.8	20.8	3-Feb	2.9
	Active metabolites	Yes	No	No	Yes (minor)	Yes	Yes
	T_{max} (hr)*	2-3	0.5-1	0.9-1.6	3	1.3-2.4	2-4
	C_{max} (ng/mL)*	27-66	448	45-55	37	10-34	10-20
	Protein binding (%)*	80-90	>99	43-55	88	94-98	>95
	Fecal excretion (%)*	70	90	71	90	58	83
	Urinary excretion (%)*	2	6	20	10	13	10

T_{max} = Time to peak concentration, C_{max} = maximum concentration, F = % inhibition at 1 mg/kg, * = based on 40 mg oral dose, † = all thermodynamic properties are in kcal/mol at 25^{O}C, ‡ = calculated in % at 20 mg dose. Ref. [5, 18, 27, 28, 54-62].

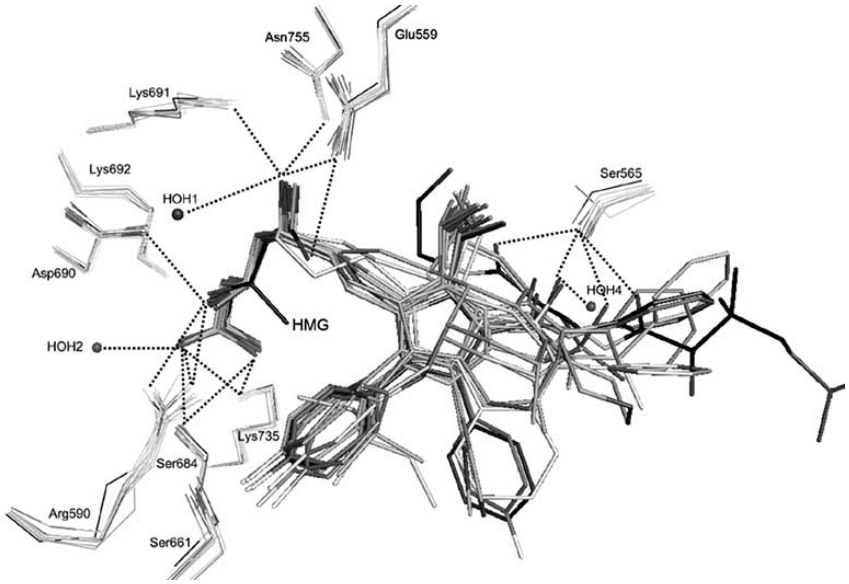


Fig. (2). The superimposed figure of statins from crystal structures of PDB code 1DQ8, 1HWK, 1HW9, 1HWL, 1HWI, 1HW8, 1HWJ, 2Q1L, 2Q6B, 2Q6C, and 2R4F are shown in different shades. For reference, substrate is also shown (carbon atoms in black). Common interacting binding site residues of human HMGR and three water molecules are also shown.

vastatin forms a strong H-bond with the hydroxyl of Ser565.

- A favorable cation-π interaction (shown as an arene-cation interaction in Fig. (**3**)) between Arg590 and the p-fluorophenyl ring is also observed in the statin structures that contain this ring.
- Various hydrophobic interactions are also observed with residues Leu562, Val683, Ala751, Leu853, Ala856, and Leu857 (Fig. (**3**)).
- In addition to protein residues, most of the inhibitors also show H-bonds with water molecules at the carboxylate end that may help in stabilizing the statin conformation. One of these water molecules also makes solvent contacts with Asp690 in many of the statin structures.
- Bacterial enzymes also show a similar pattern of interactions, both with substrate binding and the inhibitor binding (PDB codes 1QAX and 1T02, for substrate and inhibitor lovastatin, respectively).

2.3. Side Effects

Although statins generally are regarded as safe and well tolerated drugs, adverse effects have been reported. These side-effects range from skeletal muscle-related toxicity (myalgias, rhabdomyolysis) and cataracts to vascular lesions in the central nervous system (CNS) and testicular degeneration [64-69].

Since not all statins elicit the same kind or intensity of response, either in lowering lipids or in their side effects, it has been suggested that these differences from their distinct

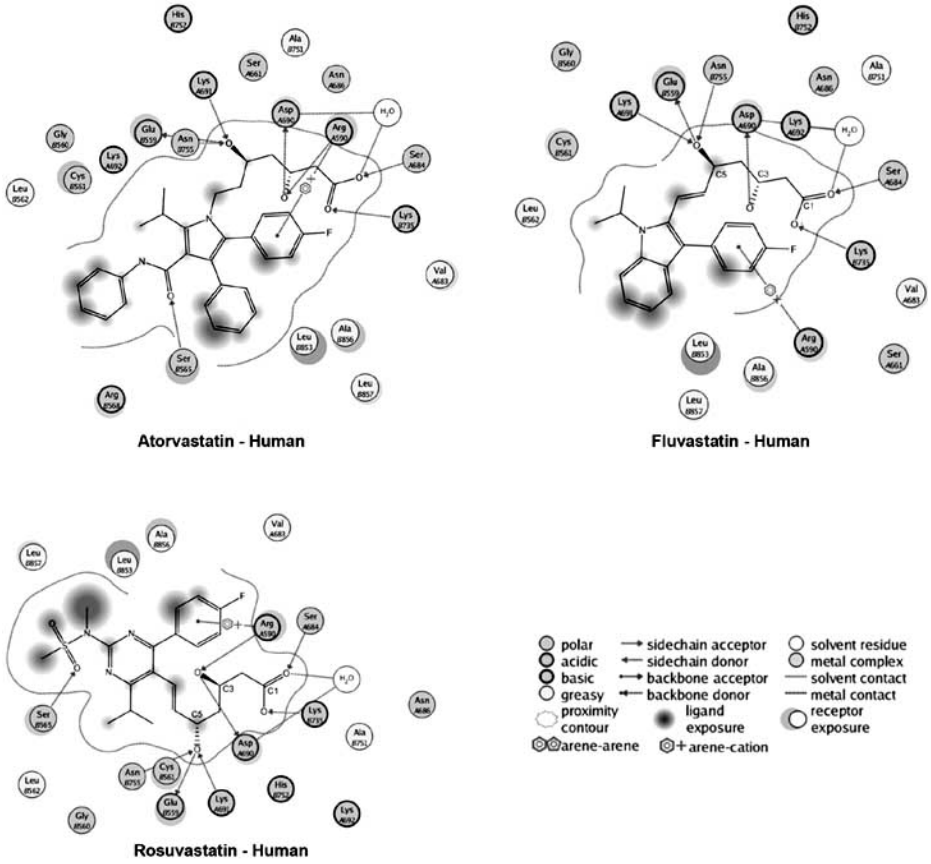


Fig. (3). Comparison of the various statin-binding sites from human enzyme HMGR. The residues are numbered according to the numbering in crystal structures of PDB code 1HWK (Atorvastatin), 1HWI (Fluvastatin), and 1HWL (Rosuvastatin). Figure legend is shown in lower right corner.

chemical structures of the non HMG-like part could vary in the presence of different functional groups, and differ in their size, the number of carbon atoms, and the number and type of polar atoms. These structural differences cause them to bind to unwanted targets, alter associated downstream metabolic pathways and produce unsafe side products, etc. [54]. While some of these fortuitously beneficial side effects called 'pleiotropic' effects (plaque stabilizing, anti-inflammatory, and antithrombotic, endothelial, and immunomodulatory effects, etc. [the following selected reviews have detailed about these effects [70-80], another (rhabdomyolysis) is fatal and led to the withdrawal of cerivastatin from the market in 2001 [81, 82].

The relative lipophilicity of statins, which is markedly different due to the presence or absence of polar moieties on the largely hydrophobic backbone, plays a major role in their pharmacokinetic profile and hence therapeutic utility. Among the statins, lovastatin, simvastatin, atorvastatin, and fluvastatin are classified as lipophilic, while pravastatin and rosuvastatin are classified as more hydrophilic and ranked as follows in decreasing order of lipophilicity: simvastatin > fluvastatin/atorvastatin > lovastatin > pravastatin > rosuvastatin. This important distinction is linked to the presence of a hydroxyl or methyl group on the hydrophobic backbone. This property also results in a differential physical distribution of the statins within the phospholipid bilayer of the cell membranes and hence determines their hepatoselectivity profile, which has also been linked to their associated side effects. Rosuvastatin, which is the most potent and hydrophilic statin available (log D = -0.33), has been shown to be the most liver-selective compared to lipophilic statins, simvastatin and cerivastatin (log D > 1.5) [83, 84]. Small-angle X-ray diffraction experiments have shown that the more hydrophilic statins are associated with the hydrated, polar surface of the membrane, whereas the more lipophilic goes much deeper into the membrane and can make hydrophobic interactions with the phospholipid acyl chains [58, 85]. In accordance with these experiments, the myotoxic and rhabdomyolytic effects of cerivastatin have also been linked to its membrane location and associated with the terminal regions of the fatty acid chains [58, 60]. Since the more lipophilic statins penetrate better into the hepatocytes, some hepatoselectivity is observed from all of them. These statins can enter cells passively and non-selectively by diffusion, and they can also diffuse out rapidly, a property that can contribute to both their side and pleiotropic effects. In comparison, the more hydrophilic statins are transported into the liver by a 'one way' carrier-mediated active transport system involving an organic anion transporter polypeptide (OATP). These statins tend to stay in the hepatic cells because of their hydrophilicity and hence do not get distributed outside *via* passive diffusion. This is the reason why these statins do not

penetrate well into the cells of other tissues like myocytes and have reduced side effects [86, 87].

The membrane locations of statins also correlate well with the differences in their metabolic fate and antioxidant effects. Simvastatin, lovastatin, and atorvastatin, which are located in the membrane hydrocarbon core (adjacent to the phospholipid headgroups) are all metabolized through oxidation by the same membrane-bound cytochrome P450 enzyme (CYP3A4 is its predominant isoform) [88]. In contrast, pravastatin (located on the membrane surface) and rosuvastatin (located in a unique position in the phospholipid headgroup/glycerol backbone region) have separate metabolic pathways and are mostly eliminated without modification. Moreover, the fact that the elimination half-lives of more complex statins, atorvastatin and rosuvastatin, are substantially longer compared with other statins (due to aromatic hydroxylation of phenyl rings), contributes to their greater efficacy in lowering LDL-C [85].

The non HMG-like moiety of the statins also determines, to a large extent, their different thermodynamic behavior, as explored by isothermal titration calorimetric (ITC) experiments. Except for fluvastatin, the binding affinity of all tested statins is characterized by favorable binding enthalpies ranging from -2.5 to -9.3 kcal/mol at 25 °C, with entropy being the dominant contribution. Only for rosuvastatin, which showed the strongest binding enthalpy, does the enthalpy change contribute more than 50% of the total binding energy (76%). At a physiological temperature of 37 °C, the binding enthalpy contributes close to 100% of the binding energy of rosuvastatin and only 42%, 44%, and 57% of that of pravastatin, cerivastatin, and atorvastatin, respectively. Overall these thermodynamic properties depend on: the amount of polar and non-polar surface that is buried in binding (highest for atorvastatin); additional H-bonds and van der Waals interactions (highest for atorvastatin and rosuvastatin); atom types involved in H-bonds (like the sulfonyl group of rosuvastatin, which gives strong favorable binding enthalpy values); and the flexibility of compounds i.e., number of rotatable bonds which determines the conformational entropy term of binding affinity (highest for atorvastatin). These properties are directly linked to the affinity of the statins with HMGR [54, 56].

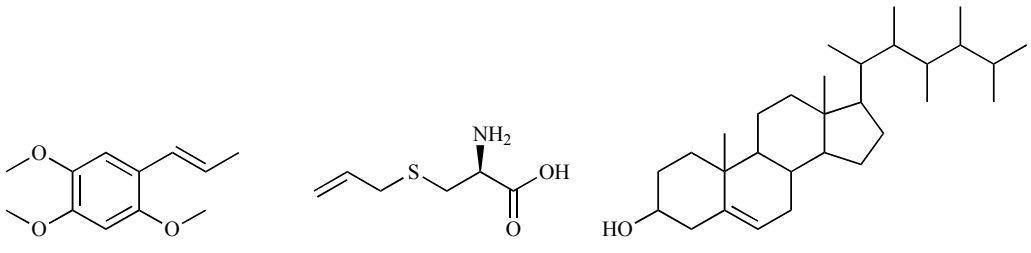
2.4. Non-Statin HMGR Inhibitors

There are various other chemical compounds that are known to inhibit HMGR, such as: α-asarone (described in next paragraph), β-sitosterols; [89] policosanol; [90] cholestin; [91] diosgenin; [92] *S*-allyl-, *S*-ethyl-, and *S*-propyl-cysteines of garlic extracts; [93] rice bran oil extract γ-oryzanol and tocotrienols; [94, 95] fermented milk products; [96] Korean soybean isoflavones; [97] SMase C generated ceramide; [98] green and black tea extracts; [99] analogues of farnesyl pyrophosphate like farnesyl acetate and ethyl farnesyl ether; [100] ketanserin tartrate; [101] L-triiodothyronine; [102] cysteine protease inhibitors like *N*-acetyl-leucyl-leucyl-norleucinal and *N*-acetyl-leucyl-leucyl-methioninal; [103] tunicamycin; [104] oxylanosterols like 25-hydroxylanosterol and 25-hydroxylanostene-3-one; [105] Lanosterol analogue 15-*alpha*-fluorolanost-7-en-3-*beta*-ol; [106] vitamin D3 derivatives, [107] and SR-12813 [108]. Structures of α-asarone, *S*-allyl-cysteine, and β-sitosterol, as examples of structurally diverse non-statin HMGR inhibitors, are shown in Fig (**4**). No experimental HMGR structures are available for any of the bound non-statin inhibitors.

α-Asarone is the active principle of the medicinal plant yumel (*Guatteria gaumeri* Greenman) with potent hypolipidemic activity [109]. α-Asarone is isolated from a variety of plants like *Acorus calamus* [110] and *Asarum europaeum* [111] and has traditionally been used for lipid lowering in the Yucatan peninsula of Mexico. It has been established that α-asarone inhibits hepatic HMG-CoA reductase [112]. Using an automated docking approach, we have reported a binding model of α-asarone with HMGR, concluding that the three oxygen atoms of this natural product contains a HMG-like moiety [109]. In order to improve the activity and pharmacological profile of α-asarone, numerous synthetic analogues have been prepared [113-118] revealing pharmacophoric groups for its activity. A structure-based design program is on-going in our group.

2.5. Computational Studies

The recently published crystal structure of statins (Table **1**) has boosted the structure-based design of novel compounds, molecular modeling of statins and application of other computer-aided drug design techniques. For example, the crystal structure of fluvastatin was the starting point of a three-dimensional (3D) quantitative structure-activity relationship (QSAR) study of 29 imidazolyl and *N*-pyrrolyl heptanoates [59]. Another independent 3D-QSAR study was performed with 35 statins and statin-like compounds. These QSAR models were used to conduct a virtual screening for potential inhibitors. As a result, eight non-statin-like compounds with potential activity have been proposed [119]. A QSAR study has been carried out for a large number chiral and diverse HMGR inhibitors using topological indices [120]. The crystal structure of cerivastatin, rosuvastatin and



α-Asarone *S*-allyl-cysteine β-Sitosterol

Fig. (4). Structure of selected and structurally diverse non-statin HMGR inhibitors.

atorvastatin bound to HMGR were also used to do docking and virtual screening of a large database of lead-like molecules, and putative HMGR inhibitors were proposed [121]. The structure-based drug design of 'pyrrole-based, [24, 27, 122] pyridine and pyrimidine-based [123, 124] and quinoline-based [125]' analogues has also been done in recent years. Finally, molecular modeling studies have also been employed to design competitive peptide-based inhibitors for HMGR [126-129].

3. CONCLUSIONS AND PERSPECTIVES

The use of statins has proven to be one of the most successful and effective approaches for the treatment of elevated plasma LDL-C levels in hypercholesterolemia and mixed dyslipidemia. In recent years, the availability of structural data has provided scientists with a better understanding of how statins bind and inhibit the function of HMGR, thus inhibiting the cholesterol biosynthesis pathway.

Though, statins have been in use for more than two decades, myotoxicity is still an issue with varying degree, depending on the statin in use. The current focus of many research groups is to design novel statins, or modify the known statins to enhance hepatoselectivity and hence improve muscle safety profile. At present, the most hepatoselective compound is rosuvastatin, with a ratio of hepatocyte IC_{50}/L6 IC_{50} of 926. Lower selectivity occurs with cerivastatin (ratio of 4.1), simvastatin (115), pravastatin (444) and atorvastatin (144) [24]. Improving hepatoselectivity may help reduce the observed side effects of myalgia by lowering availability to myocytes [130]. The rationale is that, unlike lipophilic compounds, more polar moieties on the hydrophobic backbone will create more enthalpically driven binding through electrostatic and H-bonding interactions, which potentially would diminish the cell permeability of statins in myocytes and hence result in improved cell selectivity [27, 28, 122, 131].

Finally, dissecting the mechanism of HMGR inhibition has created new ways for treatments of cholesterol related illness, and has opened new possibilities for both computational and experimental drug-design strategies such as the structure-based design of α-asarone analogues.

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ABBREVIATIONS

CVD	=	CardioVascular Disease
WHO	=	World Health Organization
HMG-CoA	=	3-Hydroxy-3-Methylglutaryl Coenzyme A
HMGR	=	3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase
LDL	=	Low Density Lipoproteins
NAD(P)H	=	Nicotinamide Adenine Dinucleotide Phosphate
ITC	=	Isothermal Titration Calorimetry
OATP	=	Organic Anion Transporter Polypeptide
QSAR	=	Quantitative Structure-Activity Relationships

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