Characterization, Modes of Synthesis, and Pleiotropic Effects of Hypocholesterolemic Compounds – A Review

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Abstract: Studies on the various cholesterol-lowering agents is one of the important areas in clinical research. Identification and characterization of potential molecules from various sources have been carried out in the past and their relationship with the enzymes which are involved in the cholesterol cascade is gaining interest. In this review, we have highlighted various inhibitors involved in the cholesterol cascade as well as cholesterol-lowering agents, *viz.*, tocotrienol, flavonoids, phytosterols, phytostanols, statins, DADS, and synthetic compounds. The mechanism of action and characterization of these hypocholesterolemic compounds are discussed in this communication. Major natural sources as well as synthetic and biological routes of synthesis of these compounds are reviewed in a concise manner. Especially, various HMG-CoA analogues including statins have been reviewed specifically.

Keywords: Hypocholesterolemic compounds, HMG-CoA reductase inhibitors, Tocotrienol, Flavonoids, Phytosterols, Statins, DADS.

INTRODUCTION

Imbalance of cholesterol level leads to hypercholesterolemia, a predominant risk factor for atherosclerosis and associated coronary and cerebrovascular diseases, as a result of environmental, genetic factors, and food habits. Cardiovascular diseases are mainly reported to cause mortality with the patients who have high serum cholesterol level especially low density lipoprotein cholesterol (LDL-cholesterol), very low density lipoprotein cholesterol (VLDL-cholesterol), and triglycerides. Body also exhibits its own natural regulation mechanisms to control cholesterol synthesis. One of the short-term regulations of the cholesterol biosynthesis cascade is done by the phosphorylation of the glycoprotein, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) using AMP-dependent protein kinase [1, 2].

In this respect, researchers have identified 2,3-oxidosqualene cyclase-lanosterol synthase (lanosterol synthase, oxidosqualene-lanosterol cyclase, lanosterol synthase, 2,3-oxidosqualene-lanosterol cyclase, human lanosterol synthase (EC 5.4.99.7)) having a molecular weight of 83 kDa, catalyzing the highly selective cyclization reaction from the substrate 2,3-oxidosqualene (squalene 2,3-epoxide, squalene 2,3-oxide, (S)-squalene-2,3-epoxide, 2,3-epoxisqualene, oxidosqualene) into lanosterol, as an appropriate step for the inhibition of cholesterol biosynthesis [3]. Oxidosqualene cyclase inhibitors (OSCI) arrest the downstream of 2,3-oxidosqualene which helps to stimulate epoxysterols to repress HMG-CoA reductase expression [4]. Some examples for this group of drugs are Ro 48-8071, 2,3:22,23-dioxido squalene and 24,25-epoxycholesterol, 4-piperidinopyridine, 4-piperdinopyrimides, quinuclidine, and umbelliferone aminoalkyl derivatives [3-7]. The progress in identifying the different sites of inhibition in the cholesterol cascade will be discussed separately.

Researchers have always tried to find effective inhibitors of HMG-CoA reductase for the treatment of hypercholesterolemia. HMG-CoA reductase is the primary target in the current clinical treatment of hypercholesterolemia. Two-third of the body's cholesterol is synthesized in the liver with the help of HMG-CoA reductase as the rate-limiting enzyme of the mevalonate pathway for cholesterol biosynthesis [8]. Detailed studies on HMG-CoA reductase would help the researchers to develop novel inhibitors with more affinity compared to that of statins. Bochar *et al.* (1999) established the crystallographic analysis of abortive ternary complexes of this enzyme and reported that lysine-267 located at a position in the active site might act as the general acid/base for catalysis. Therefore, site-directed mutagenesis and subsequent chemical derivatization were employed to investigate the active site and the replacement of lysine-267 by alanine, histidine, or arginine, resulting in mutant enzymes that lack detectable activity [9]. The existence of two distinct classes of the enzymes, called Class I and Class II HMG-CoA reductase were revealed using the genome sequencing technique. This categorization was based on the comparison of the sequences of eukaryotic, prokaryotic, and archaeal HMG-CoA reductases [1, 9]. Histidine 381 of the HMG-CoA reductase from *Pseudomonas mevalonii* was identified as the main residue functional in catalysis [10]. For the HMG-CoA reductase of *P. mevalonii*, the catalytic domain contains three conserved acidic residues, *viz.*, Glu 52, Glu

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83, and Asp 183 [11, 12]. Tabernero *et al*. [13] reported the crystal structure of a lovastatin bound to both class I (human) and class II (bacterial) HMG-CoA reductases. This work gives an impetus for the development of selective class II inhibitors for use as antibacterial agents against pathogenic microorganisms.

Numerous reports are available on the synthesis of chemical compounds to control the metabolites of the cholesterol cascade when the natural body regulation fails to control the cholesterol level. One of the groups of such compounds is called statin, also known as HMG-CoA reductase inhibitors. These compounds are structurally analogous to HMG-CoA and exhibits competitive inhibition with HMG-CoA reductase. This enzyme catalyzes the rate-determining step of mevalonate formation from HMG-CoA in the *de novo* cholesterol synthesis pathway. The downstream of mevalonate, terminated by these drug molecules leads to pleiotropic effects [8, 14, 15]. Some fungi produce group 1 statins as secondary metabolites. A few organisms have been employed for biotransformation studies to improve the efficiency of fermentation-derived statin compounds [2, 15, 16]. Differences in structure and binding characteristics partly contribute to differences in the inhibition efficiency of statins. This results in variation in the reduction of undesirable cholesterol levels and other pharmacological properties. These drugs are commonly prescribed for coronary patients and it has been found to reduce the LDL-cholesterol and triglyceride levels and increase the HDL-cholesterol in the patients [17, 18]. Though statins are considered generally safe, they are known to cause myopathy and in rare cases rhabdomyolysis. Evidence suggests that myotoxicity is due to inhibition of HMG-CoA reductase within the myocyte [19]. Thus, an inhibitor with increased selectivity for hepatocytes could potentially result in an improved therapeutic window with fewer side effects. The necessity to find an alternative approach is due to the unfavorable effects of statins.

The addition of specific functional groups to fermentation-derived statins and the synthesis of new compounds have received more importance in clinical research. Compounds like hydroxyl phosphinyl trans-tetrahydro compactin [20], tocotrienol (a vitamin E analogue) [21], 4-thiophenyl quinoline-based mevalonolactones [22], α,α-difluoroketones [23], and daidzein (isoflavone) [24] are some of the HMG-CoA reductase inhibitors. Farnesylated benzopyran (tocotrienols) among other compounds were found to suppress the post-transcription of HMG-CoA reductase (Table **1**). Apart from the HMG-CoA analogues, some compounds, *viz*., the fiber β-gulcan, plant sterols, and oxygenated terpenes were also found to have hypocholesterolemic effect [16]. Red yeast rice (*Monascus* fermented rice), some cereal grains, rice bran, garlic (*Allium sativum*), and barley were found to reduce cholesterol level. Later, it was found that tocotrienols, diallyl disulfide analogues, daidzein, and some of their analogues were responsible for the hypocholesterolemic effect (Table **1**). There could be more promising molecules exhibiting hypocholesterolemic effect which need to be explored.

Torcetrapib is a known inhibitor of cholesterol ester transfer protein (CETP), which increases high density lipoprotein (HDL) cholesterol, though it increases the frequency of coronary problems. The actual function of CETP is to promote the transfer of cholesteryl ester from antiatherogenic HDLs to proatherogenic apolipoprotein B (apoB). The inhibition of this protein leads to an increase in the HDL-cholesterol level and a decrease in the LDL-cholesterol level, which is desirable [48, 49].

There are some more synthetic compounds which exhibit hypocholesterolemic activity. This article reviews the different molecules involved in the inhibition of cholesterol absorption in guts, regulation of serum cholesterol level, and cholesterol synthesis in liver. Various HMG-CoA analogues, their mechanisms of action, routes of synthesis, and pleiotropic effects have been reviewed which could probably rekindle interest in the discovery of new molecules displaying hypocholesterolemic activity and the mechanisms of their action.

CHARACTERIZATION AND MECHANISMS OF HYPOCHOLESTEROLEMIC COMPOUNDS

Various hypocholesterolemic compounds such as tocotrienols, diallyl disulfide (DADS), flavonoids, 5-substituted 3, 5-dihydroxypentanoic acid and its analogues, plant sterols and statins, their characterization, and modes of action are summarized in Table **1**. Inhibition of cholesterol synthesis, gene expression, cholesterol uptake rate, and molecular signaling are typical ways by which these compounds control cholesterol level.

Tocotrienols are farnesylated benzopyran, one of the basic constituents of vitamin E. They are structurally similar to tocopherols and differ mainly by the presence of unsaturation (three double bonds) in the isoprenoid side chain, essential for the inhibition of cholesterogenesis. There are four different types of tocotrienols (α-, β-, γ-, and δ-) present in nature (Fig. **1A**) [50]. They differ from each other due to (i) their protective mechanism against cell toxicity, (ii) their physical effects due to radical scavenging action at the interior of the liposomal membrane, and (iii) their radical scavenging efficiencies and chemical reactivity with metal ions in solution. Among the four types, the γ-tocotrienol was found to have significant clinical property and it exhibited a 30-fold greater activity compared to α-tocotrienols [16, 51]. These molecules exhibited hypocholesterolemic activity under both *in vitro* and *in vivo* conditions (Table **1**). Tocotrienols suppress the post-transcription of HMG-CoA reductase and their side-chain's unique ability to increase cellular farnesol (a mevalonate-derived product) signals the proteolytic degradation of HMG-CoA reductase. Both the natural and synthetic tocotrienols are equally active in the inhibition of the cholesterol cascade as well as the suppression of HMG-CoA reductase expression. Desmethyl tocotrienol (3,4-dihydro-2-methyl-2-(4,8,12-trimethyltrideca-3′(E),7′(E),11′-trienyl)-2H-1-benzopyran-6-ol) and didesmethyl tocotrienol (3,4-dihydro-2-(4,8,12-trimethyltrideca-3′(E),7′(E), 11′-trienyl)-2H-1-benzopyran-6-ol) are two novel tocotrienols which exhibited high suppression of total serum and LDL-cholesterol levels and these were obtained from stabilized rice bran [25, 26]. The number and position of methyl substitutions in the tocotrienols leads to some of their typical properties, *viz*., hypocholesterolemic and antioxidant activity [16, 27, 51].

Table 1. Different Hypocholesterolemic Compounds and their Characteristics

Source	Compound	Type of Inhibition	Remarks	Mode of Action	Reference
Hevea brasiliensis, vegetable oils (palm oil (rich), wheat germ, bran, meal, flour, barley, saw palmetto, and certain types of nuts and grains.	**γ-, β-, δ-, α-Tocotrienol**	Represses HMG-CoA reductase expression	Degradation rate of 2.3 fold per 10μg of γ-tocotrienol	Both *in vitro* and *in vivo*	[16, 25-27, 28]
Garlic (*Allium sativum*).	**Diallyl disulfide analogues**	Inhibition of sterol regulatory element-binding proteins (SREBPs), cAMP response element-binding protein (CREB) which are responsible for the expression of HMG-CoA reductase	Effect on HDL, LDL, Triglycerides, SREBP-2, CREB and nuclear factor-Y (NF-Y)	Maximum activity at *in vivo* and moderate activity at *in vitro* conditions	[2]
Buckwheat (*Fagopyrum esculentum* Moench), *Pterocarpus marsupium*, cabbage, apple, red wine, citrus, graph and tea.	**Flavonoids** (Naringin, rutin, marsupsin, pterosupin, and liquiritigenin)	Inhibition of lipid peroxidation	Effect on total cholesterol, HDL, LDL, triglycerides. R_f value (rutin) 12.4 min. daily doses of 180 to 350 mg. Trolox equivalent antioxidant activities (TEAC) 2.4 (rutin) and 0.24 (naringin). Peak time around 2 h and $t_{1/2}$ (20-72 h).	*in vitro*	[29-33]
Pueraria thunbergiana, soya beans.	**Isoflavone** (daidzein, genistein, glycitein, aglycone form)	Competitive inhibition to HMG-CoA reductase and non-competitve inhibition with respect to NADPH	IC_{50} = 4.25 μM , K_m = 32±6 μM K_i = 27.7, 49.5, and 94.7 μM for genistein, daidzein, and glycitien, respectively.	*in vitro*	[24, 34, 35]
Pholiota adipose (mushroom).	**Phytosterols** stigmasterol	Inhibition of HMG-CoA reductase	LD_{50} = 6.8 μg/g inhibition activity = 55.8%	*in vitro*	[36, 37]
nuts, seeds, unrefined plant oils and legumes.	**Unsaturated phytosterols** (stigmasterol, brassicasterol, and ergosterol)	Inhibition of sterol Δ^{24}-reductase	K_i = 41.1, 42.7, and 36.8 μM K_m (desmosterol) = 26.3 μM		[38, 39]
Aspergillus (A. terreus, A. flavus, etc.), Monascus (M. purpureus, M. ruber, M. pilosus, M. anka etc.), Pencillium (P. cyclopium, P. brevicompactum, P. citrinum etc.), Streptomyces sp. etc.	**Lovastatin** (LV) **Simvastatin** (SV), **Pravastatin** (PV), **Mevastatin** (MV), **Wuxistatin** (WS), **Fluvastatin** (FV), **Rosuvastatin** (RV), **Atorvastatin** (AV).	Inhibition of HMG-CoA reductase	K_i (nM), IC_{50} (nM) values: 0.6, 11.2 (LV) 0.12, 44.1 (SV) 2.3, 27.6 (PV) 0.3, 0.6 (FV) 1.45, 0.16 (MV) 0.1, - (RV) -, 8.2 (AV) -, 160 ± 10 (WS)	*in vitro*	[15, 40-46]
Synthetic	5-substituted 3,5-dihydroxypentanoic acids and their derivatives	Inhibition of HMG-CoA reductase	IC_{50} values are 22, 10.8 μM, respectively.	*in vitro*	[47]

$t_{1/2}$ = half-life period, IC_{50} = half maximal inhibitory concentration, LD_{50} = lethal dose or median lethal dose, K_i = inhibition constant, and K_m = Michaelis-Menten constant, - not reported.

Diallyl disulfide (4,5-dithiaocta-1,7-diene), an organosulfur compound in garlic (*Allium sativum*) (Fig. **1B**), is unstable and highly volatile in nature. It has anti-atherosclerotic and antioxidant properties. Their analogues inhibit the cholesterol cascade (Table **1**). These compounds inhibit the activation of sterol regulatory element binding protein-2 (SREBP-2) and cAMP response element-binding proteins (CREB), which are responsible for the expression of some enzymes involved in the cholesterol cascade especially HMG-CoA reductase [2].

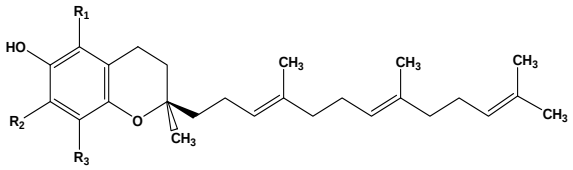


Fig. (1A). General configuration of tocotrienols [26].

	Functional group		
	R_1	R_2	R_3
α-Tocotrienol	CH_3	CH_3	CH_3
β-Tocotrienol	CH_3	H	CH_3
γ-Tocotrienol	H	CH_3	CH_3
δ-Tocotrienol	H	H	CH_3

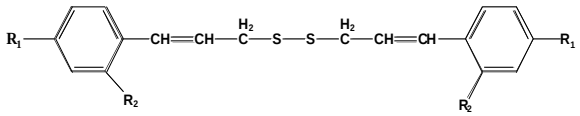


Fig. (1B). Structure of diallyl disulfide (DADS) analogues [2].

DADS (diallyl disulfide) analogues	Functional group	
	R_1	R_2
1	NO_2	H
2	H	Cl
3	Cl	Cl
4	H	Br
5	Br	Br
6	F	H
7	CF_3	H

Flavonoids are pigments fenolics of plants that possess several biological activities (Fig. **2A**). They are associated with the prevention of hyperlipidemia and cancer [29]. Naringin (a flavanone) and rutin (quercetin 3-rhamnosyl-glucoside, a flavone) are typical flavonoids which inhibit the cholesterol cascade by inhibiting the lipid peroxidation (Figs. **2A** and **2B**) [29, 30, 33]. Ethanol extract of the heartwood of *Pterocarpus marsupium* contained flavonoids, *viz*., marsupsin, pterosupin, and liquiritigenin (Table **1**) and these show a significant reduction in serum triglyceride, total cholesterol, LDL-, and VLDL-cholesterol levels. Flavonoids do not show any significant effect on the level of HDL-cholesterol in Triton®-induced hyperlipemic rats [31]. The Trolox® equivalent antioxidant activities (TEAC) value determines the anti-oxidant potency of a particular flavonoid, which decreases for a glycosidic-substitution compound. The TEAC for rutin and naringin are 2.4 mM and 0.24 mM, respectively [38]. Natural flavonoids have poor solubility and stability in hydrophobic solvents and were stabilized by the enzymatic acylation process. The resultant products are more stable and soluble in the lipophilic medium [33]. The chemical structure of flavonoids determines the degree of inhibition of the free-radical mediated mechanisms. These compounds differ in radical scavenging and chelating activity from each other based on the flavan nucleus, the number, positions, and types of substitutions [32].

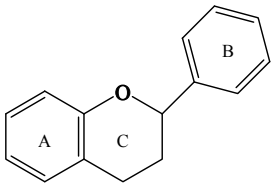
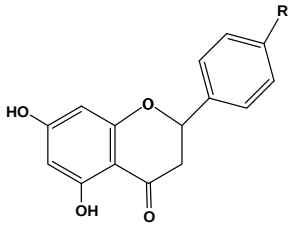


Fig. (2A). General structure of flavonoids [32].



Pinocembrin (R=H), Naringenin (R=OH)

Fig. (2B). General structure of flavanones [60].

In this flavonoid group, isoflavone is prominent for its inhibitory activity against HMG-CoA reductase. These molecules are not soluble in water but soluble in highly polar solvents like ethanol and methanol. Among isoflavones, the daidzein (4′,7-dihydroxyisoflavone) is an important compound (Fig. **2C**) exhibiting competitive inhibition to HMG-CoA reductase. Daidzein has a molecular weight of 254 Da and the half maximal inhibitory concentration (IC_{50}) was 0.9 μg (4.25 μM) against HMG-CoA reductase (Table **1**). Though it exhibited competitive inhibition to HMG-CoA reductase, the structure of daidzein cannot be compared with statin structure [24].

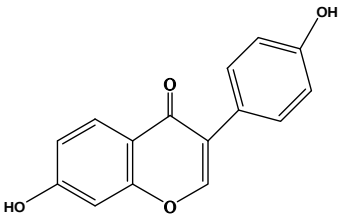


Fig. (2C). Structure of Daidzein [24].

The choice of solvents has received a significant role in the extraction of isoflavones. The activity of isoflavones heavily depends on the extraction solvent and the extraction strategy. This compound exhibited maximum HMG-CoA reductase inhibitory activity (IC_{50} = 79 μg) when 70% ethanol was used for its extraction from *Pueraria thunbergiana*. The absorption spectra of the purified HMG-CoA reductase inhibitor (daidzein) were obtained at 221 and 238 nm in methanol [24]. Some isoflavones found in soyabean paste exhibited the same HMG-CoA reductase inhibition. They are genistein, glycitein, and aglycone (Table **1**) [34, 35].

Some plant sterols (phytosterols) display both hypocholesterolemic effect and sterol (Δ^{24}) reductase inhibitory activity. These compounds reduce the synthesis of cholesterol in liver and reduce the cholesterol level in serum by inhibition or by reducing the uptake rate of cholesterol. The methanol

extract of the mushroom, *Pholiota adipose*, showed a high inhibitory activity of 55.8% on HMG-CoA reductase. Lee *et al.* found that the stigmasterol ($C_{29}H_{46}O$, M.W. 412.7 Da) was responsible for the inhibitory action and the lethal dose (LD_{50}) was 6.8 μg/g of sample. Stigmasterol, a derivative of sitosterol is poorly absorbed than sitosterol in the intestines [36]. More studies are necessary to determine the beneficial effects of the phytosterols as cholesterol inhibitors. Phytosterols reduce intestinal cholesterol absorption as well as increase the HDL-cholesterol level by still unknown mechanisms. The increase in the activity of the ATP-binding cassette transporter (ABC) A1 has been proposed as a mechanism to account for the hypocholesterolemic effect. Later, Calpe-Berdiel *et al.* have shown that this mechanism is independent of the ABC transporter system [38]. The C-22 unsaturated phytosterols, *viz*., stigmasterol, brassicasterol, and ergosterol are known competitive inhibitors of sterol Δ^{24} -reductase (lanosterol reductase or desmosterol reductase), with K_i values of 41.1, 42.7, and 36.8 μM, respectively (Figs. **2D** and **2E**). The estimated K_m for desmosterol is 26.3 μM. These sterols have a double bond at the C-22 position in the side chain and it is responsible for their significant inhibition activity against sterol Δ^{24} -reductase. This inhibitory action was confirmed by measuring the accumulated sterol precursors (mainly desmosterol) using ^{14}C-labelled desmosterol as the substrate in rat liver microsomes. There are many reports on the potential inhibitors of sterol Δ^{24} -reductase. The sterol 5,22-cholestedien-3β-ol (an unusual desmosterol isomer that lacks the alkyl groups) also appears to be a potential inhibitor of sterol Δ^{24} -reductase with a K_i of 3.34 μM [39].

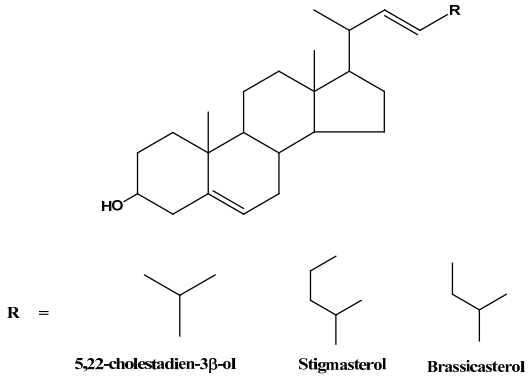


Fig. (2D). General structure of C-22-unsaturated phytosterols [39].

A series of 5-substituted 3, 5-dihydroxypentanoic acids and their lactone derivatives have been synthesized synthetically and studied for the inhibition of HMG-CoA reductase. An enantiomer of the ring-opened form of lactone ((*E*)-6-[2-(2,4-Dichlorophenyl)ethenyl]-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-one (Fig. **3A**) displays better inhibition activity of HMG-CoA reductase in *in vitro* conditions among all the combinations studied and has a melting point around 148-150^{0}C. This compound exhibited a percentage inhibition of 84 at a concentration of 20 μg/mL. The carboxylate anion formation and hydroxyl group substitution were found to increase the inhibition activity. The insertion of a bridging unit other than ethyl or an (*E*)-ethenyl between the 5-carbinol moiety and an appropriate lipophilic moiety attenuates the activity. Other derivatives which have different inhibition activities help to determine the essentiality of the functional group [47].

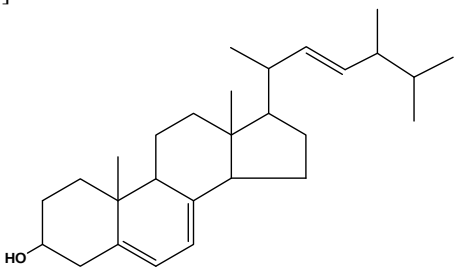


Fig. (2E). Structure of Ergosterol [39].

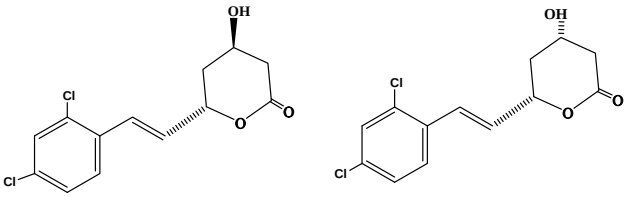


Fig. (3A). 5-Substituted 3,5-dihydroxypentanoic acids and its derivatives ((*E*)-6-[2-(2,4-Dichlorophenyl)ethenyl]-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-one) [47].

Statins, the major class of hypocholesterolemic drug, exhibit competitive inhibition with HMG-CoA reductase. Statins have a hexahydro-naphthalene ring structure and β-hydroxy-lactone. They are soluble in solvents like methanol, ethanol, chloroform, etc. and are insoluble in water. Based on the type and number of binding interactions, statins are further divided into two groups, *viz*., Group 1 (fermentation-derived) statins (simvastatin, lovastatin, compactin, and pravastatin) which exhibit binding *via* a decalin ring structure (HMG-CoA like moiety) and Group 2 (synthetically-derived) statins (rosuvastatin, atorvastatin, fluvastatin, and cerivastatin) which exhibit additional binding *via* their fluorophenyl group (Fig. **3B**). Group 2 statins contain additional functional group apart from the methyl substitution around the naphthalene structure which makes them more effective than the group I statins. For example, rosuvastatin and atorvastatin possess strong hydrogen bonds. A polar interaction between Arg568 side chain of the HMG-CoA reductase and the electronegative sulfone group is unique to rosuvastatin. It has been observed that the statin binds to the active site of biodegradable and NAD(H)-dependent HMG-CoA reductase. It interacts with the residues implicated in catalysis and substrate binding. It also displaced the flap domain of the enzyme which contained the catalytic residue His-381 [13, 40, 41, 52]. This binding with statin prevented HMG-CoA reductase from attaining the functional state. The change in conformation at the active site makes these drugs very effective and specific and also disables the enzyme from binding with the HMG-CoA. Thus it blocks the downstream of mevalonate in the cholesterol synthesis pathway, thereby reducing cholesterol synthesis in liver [52].

More reports are available on the screening of potential hypocholesterolemic compounds. Statins bind with HMG-CoA reductase in a reversible manner and their affinity for this enzyme is in the nanomolar range as compared to the

HMG-CoA which has affinity at the level of micromolar concentration. Statins reduce LDL-cholesterol in a non-linear fashion. The inhibition of HMG-CoA reductase determines the reduction of intracellular cholesterol, inducing the activity of a protease which slices the sterol regulatory element binding proteins (SREBPs) from the endoplasmic reticulum. The SREBPs help to increase the gene expression of the LDL receptor. The reduction of cholesterol synthesis at hepatic cells leads to an increase of the hepatic LDL receptors which in turn determine the reduction in the level of LDL-cholesterol and its precursors [40, 41].

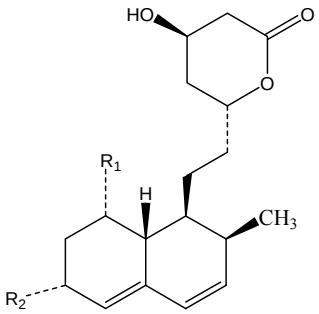


Fig. (3B). General structure of statins. R_1 is the butyric substitution in fermentation-derived statins and fluorophenyl substitution in synthetic statins, R_2 is either methyl or hydroxyl group [45].

ORIGIN AND ROUTES OF SYNTHESIS OF HYPO-CHOLESTEROLEMIC COMPOUNDS

A series of complex reaction are employed to synthesize hypocholesterolemic compounds synthetically or biologically or semi-synthetically. The sources of different hypocholesterolemic compounds are shown in Table **1**. Tocotrienol is present in natural grains, barley, and bran. They are also synthesized by the chemical route. In the biosynthesis of tocotrienols, homogentisic acid geranylgeranyl transferase (HGGT) is the key enzyme which catalyzes the first committed steps of tocotrienol synthesis. Geranylgeranyl diphosphate (GGDP) is the starting substrate condensed with homogentisic acid which gives 2-methyl-6-geranylgeranyl benzoquinol. This reaction is catalyzed by the HGGT which is found in barley, wheat, and rice seeds. Simultaneously, GGDP produces phytyl-diphosphate by its action with geranylgeranyl reductase and finally forms tocopherol. Subsequent methylation and cyclization of the 2-methyl-6-geranylgeranyl benzoquinol resulted in the synthesis of tocotrienol similar to tocopherol synthesis [53]. Chromane analogues were chosen as substrates for the synthesis of tocotrienol and were converted to tocotrienol *via* both the chemo-enzymatic and synthetic approaches. *Candida antarctica* lipase B (CALB) has been used for the stereoselective acylation of the achiral chromanedimethanol derivative with vinyl acetate to give the (*S*)-monoester and this ester subsequently formed α-tocotrienol [54].

Diallyl disulfide (4,5-dithia-1,7-octadiene) is a natural organosulfur compound found in garlic (*Allium sativum*). Diallyl trisulfide and diallyl tetrasulfide are present in the distilled oil of garlic. This diallyl disulfide can also be synthesized by the synthetic route. The sodium disulfide and allyl chloride allowed to react at 40ºC–60ºC in the presence of an inert gas finally formed diallyl disulfide as the end product [55].

Flavonoids, isoflavonoids, and neoflavonoids are natural polyphenolic compounds possessing 15 carbon atoms derived from 2-phenylchromen-4-one, 3-phenylchromen-4-one, and 4-phenylcoumarine, respectively. Flavonoids (bioflavonoids), also known as vitamin P, are available in various plants along with citrin as secondary metabolite. These flavonoids are classified and characterized based on the position of the aromatic ring attachment to the chromane ring (Fig. **2A**). They are poorly soluble in hydrophobic solvents and are modified into more stable and soluble forms in lipophilic media. The modifications typically effected on flavonoids are acylation of the glycoside moieties to yield a stable product. The presence of hydroxyl groups lead to a mixture of end products with more substitution in place of the hydroxyl group. Biocatalysts with high specificity and selectivity employed for acylation processes lead to a stable and pure product [33, 56]. The isoflavones are mostly found in soya beans and some other plants sources. The analogues of isoflavones were synthesized by specific synthetic routes, for, e.g., the Suzuki coupling reaction of 3-iodo-8-isobutyl-5,6,7-trimethoxy-2-methyl-4*H*-chromen-4-one with different arylboronic acids [57]. There is a conventional route of synthesis for isoflavones with the aid of 1,3,5-triazine as a formylating reagent, where the ring closure of 2-hydroxydeoxybenzoins lead to the formation isoflavones [58].

There are more routes to synthesize isoflavones using 2-hydroxydeoxybenzoins, for example, the formylation of 2-hydroxydeoxybenzoins with N,N-dimethylformamide. This 2-hydroxydeoxybenzoin is allowed to react with a mixture of ethyl orthoformate, pyridine, and piperidine to yield the desired compound. The isoflavones have also been synthesized *via* the decarboxylation of 2-carboxyisoflavones. The two most popular synthetic pathways to isoflavones are the deoxybenzoin (2-hydroxyphenyl benzyl ketone) and the chalcone routes [59]. Naringenin is a central precursor of many flavonoids, obtained from the phenylpropanoid pathway. The phenylalanine ammonia lyase (PAL) from *Rhodosporidium toruloides*, 4-coumarate: coenzyme A (CoA) ligase (4CL) from *Arabidopsis thaliana* and chalcone synthase (CHS) from *Hypericum androsaemum* are integrated into *Saccharomyces cerevisiae* strains to accomplish the biosynthesis of naringenin. By genetic manipulations, the phenylpropanoid pathway has been expressed in the yeast system with a maximum production of 7 mg/L of naringenin. This helps in the expression of some of the by-products such as pinocembrin (0.8 mg/L), 2',4',6'-trihydroxydihydrochalcone and phloretin [60, 61].

Phytosterols are plant sterols and stanols. They have structural similarity with cholesterol except that they always contain some substitutions at the C-24 position on the sterol side chain. These sterols can be hydrogenated to obtain phytostanols. In this process, the unsaturated phytosterols were converted to the saturated phytostanols by a hydrogenation reaction in the presence of a suitable catalyst (Pd or Pt) and a suitable solvent under high hydrogen pressure. These compounds have hypocholesterolemic property and were obtained from various sources like nuts, seeds, wood pulp industrial waste, unrefined plant oils (soyabean oil, rapeseed

oil, sunflower oil), legumes, fungi, algae, and yeast sources (Table **1**). The most common phytosterols and phytostanols are sitosterol (3β-stigmast-5-en-3-ol), sitostanol (3β,5α-stigmastan-3-ol), campesterol (3β-ergost-5-en-3-ol), campestanol (3β,5α-ergostan-3-ol), stigmasterol (3β-stigmasta-5,22-dien-3-ol), and brassicasterol (3β-ergosta-5,22-dien-3-ol) [62].

The typical composition of these compounds varies in the commercial sources. The major source of phytosterols is vegetable oil and tall oil. These crude oils are subjected to various process steps starting from the removal of impurities and ending in the final refining process. The refining process can be carried out either physically or chemically. Though the physical refining process gives less number of by-products at the end of processing, the chemical refining process has been chosen for large-scale refining. The typical refining process includes the following steps, *viz*., degumming, neutralization, bleaching, and steam-refining or deodorization. The ester forms of sterol or steryl esters can be converted into free sterols (unsaponifiable) *via* a hydrolysis process. Then it is subjected to a combined process of saponification and hydrolysis and the resultant product is recovered by vacuum distillation/evaporation. There are a number of approaches to the synthesis of phytosterols and its derivatives through a series of steps [38, 39, 62].

Reports are available on the enumeration of possible routes of biosynthesis of phytosterols [61, 62]. Isotopes have been used to track the possible routes. The major sterol of the brown algae (*Phaeophyceae*) is fucosterol. Earlier, with *Laminaria saceharina*, it has been reported that the C-24 ethylidene group of fucosterol could be derived by a double transmethylation reaction from methionine. Phytosterol biosynthesis has been examined in the marine brown alga (*Fucus spiralis*), where the transmethylation mechanism has been studied in a detailed manner using a radio assay technique [63]. Synthesis of side chain-modified phytosterols has been achieved by temporarily masking the stigmasterol 5,6-alkene, which can be regenerated *via* mild deoxygenation. By this approach, one can synthesize β-sitosterol and campesterol acetate suggesting an easy route to the synthesis of the (*Z*)-isomers of Δ^{22-23} phytosterols. Rossi *et al*. [64] synthesized phytosterol colloidal particles using a simple food-grade method based on antisolvent precipitation in the presence of a non-ionic surfactant. The resultant colloidal particles had a rod-like shape. The presence of water and hydroxyl groups on the particle surface, steric stabilization, and the presence of a non-ionic stabilizer increased the stability of the colloidal particles which was attributed to the surface charge. These prepared colloidal phytosterol particles could be effectively solubilized in model dietary mixed micelles and the micellar cholesterol concentration could be effectively reduced by 47% within 2h. The reason behind this increased reduction is due to its high solubility as evidenced in the *in vitro* assays [64].

Statins are a class of cholesterol cascade blockers which compete with HMG-CoA to competitively inhibit HMG-CoA reductase. Among the two types of statins, statins obtained by the fermentation route, *viz*., lovastatin, compactin, pravastatin, simvastatin (semi-synthetic) are produced by different organisms like *Aspergillus*, *Monascus*, *Pencillium*, Streptomyces, and *Actinomycetes spp.* as secondary metabolites. The production is associated with a series of reactions and these compounds help these organisms to protect themselves from competent organisms by blocking the membrane lipid synthesis. Studies on *Aspergillus terreus* revealed that the biosynthesis pathway of statins has two different enzymatic systems known as multifunctional polyketide synthase systems, *viz*., the lovastatin nonaketide synthase (LNKS) system involved in the cyclization of the main polyketide chain to form the hexahydro-naphthalene ring and the lovastatin diketide synthase (LDKS) system involved in the synthesis of the methylbutyryl side chain. Similarly, the additional two methyl groups at C-19 and C-24 present in compactin were derived from methionine. Studies have been carried out to find the source of the functional groups present in and around the core structure of the statins. Both lovastatin and compactin have 2-methyl butyl substitution which originates from acetate molecules. Statins like wuxistatin and simvastatin can be obtained from lovastatin, and pravastatin can be derived from compactin through biotransformation especially by *Actinomycetes sp.* There exists another class of synthetic statins (e.g. rosuvastatin, atorvastatin, pitavastatin, and cerivastatin) which are prepared by a series of chemical reactions and have low inhibition constant (K_i) values than the statins derived by the fermentation route [15, 45, 46, 65].

An enantiomer of the ring-opened form of lactone (Fig. **3A**) shows better inhibition activity (IC_{50} = 22 μM) of HMG-CoA reductase among a series of 5-substituted 3,5-dihydroxypentanoic acids and their derivatives in *in vitro* condition. It has been reported that the insertion of a bridging unit other than ethyl or (E)-ethenyl between 2,4-dichlorophenyl and 5-carbinol moiety had decreased the activity [47].

PLEIOTROPIC EFFECTS OF DIFFERENT HYPOCHOLESTEROLEMIC DRUGS

Compounds produced from plants and fungal sources have pleiotropic effects among which anti-cholesterolemy is a prominent one. Apart from anti-cholesterolemic property, these hypocholesterolemic drugs possess anti-oxidation, anti-proliferation, anti-cancer, anti-inflammatory, anti-diabetic, anti-atherosclerotic, and anti-angiogenic properties. Also, these compounds were efficacious in the treatment of cardiovascular disease (CVD), Alzheimer's disease, renal disease, cancer, bone fracture, allergic encephalomyelitis, multiple sclerosis, immune-mediated neurological disorders, and also diabetes [15, 27, 32, 35, 39, 62, 66-69]. Tocotrienols possess other potential medicinal properties apart from the hypocholesterolemic effect, and are able to reduce the atherogenic apolipoprotein B, lipoprotein(a) plasma levels. Tocotrienol is also suggested to have anti-thrombotic and anti-tumor effect, indicating its use in the prevention and/or treatment of cardiovascular disease and cancer [66]. Research on tocotrienols has suggested that these molecules are good antioxidants and, therefore, they reduce risks due to CVD and cancer by arresting free radical damage. The role of tocotrienols in the prevention of CVD and cancer may have significant clinical implications [27, 66]. Diallyl disul-

fide has antiatherosclerotic and antioxidant properties. Their analogues have the specific property of hypochloesterolemia helping to reduce the LDL-cholesterol and serum cholesterol levels by inhibiting transcriptional activators [2].

Flavonoids are also known anti-oxidants. Among this group, few flavonoids have shown hypocholesterolemic activity in addition to anti-cancer, metal-chelating and pro-oxidant activities [32, 33, 36]. Daidzein is a major isoflavone present in soyabeans and other plants, which displays important biological activities including the reduction of breast tumor formation, antioxidation, antiproliferation, antiangiogenic activity, and the inhibition of cytokinetic action and growth factors [24]. Plant sterols are also proven inhibitors of HMG-CoA reductase and sterol reductase along with other beneficial properties like anti-cancer, anti-CVD and anti-atherosclerosis [39]. Phytosterols and phytostanols are also used in food and beverage manufacture. These esters of phytosterol are present in daily consumables like yogurt (1.25 g/125 ml), spread (3.4 g/30 g), and milk (5 g/L). The addition of phytosterols and phytostanols to various food products showed excellent stability at different pH values during long-term storage. Food items exhibit oxidative stability when phytostanol esters are present as a constituent [62, 67, 68].

Statins also have pleiotropic effects. These compounds prevent Alzheimer's disease and are used in the treatment of renal disease, cancer, bone fracture, and CVD and they also increase HDL-cholesterol levels [15]. Lovastatin treatment decreased neuro-inflammatory activity and allergic encephalomyelitis, and multiple sclerosis. Statins, in addition to their lipid-lowering effects, possess anti-inflammatory and immunomodulatory properties useful in the treatment of immune-mediated neurological disorders. Lovastatin therapy can significantly reduce morbidity and mortality in diabetics [15]. Atorvastatin and rosuvastatin also have antimicrobial activity and they inhibit isoprenoid synthesis essential for the normal cell function of prokaryotes [69].

CONCLUSIONS

Various hypocholesterolemic compounds like tocotrienols, diallyl disulfide, flavonoids, 5-substituted 3,5-dihydroxypentanoic acids and its analogues, plant sterols and statins were discussed and their characterization and modes of action were also described in a concise manner. Routes of synthesis of these compounds from various sources and their pleiotropic effects were also discussed. More specifically, the naturally-derived HMG-CoA reductase inhibitors were elaborated. More hypocholesterolemic molecules need to be screened from different sources and their modes of action must be ascertained. Also, the efficiency of the existing molecules needs to be improved *via* biotransformation or synthetic routes.

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CONFLICT OF INTEREST

Probably such phenomenon may not arise in this regard.

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