



## Simvastatin: Biological Production, Therapeutic Applications and Proposed Alternative Hypothesis for Biosynthesis

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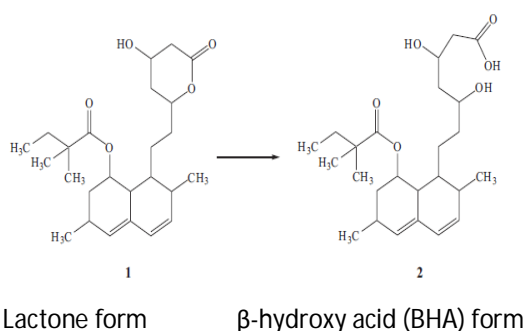
### ABSTRACT

Simvastatin is a potent inhibitor of the enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) that catalyzes the rate limiting step in cholesterol biosynthesis pathway. Simvastatin is active *in vitro* as well as *in vivo* to inhibit cholesterol synthesis. It lowers plasma cholesterol level, thus effective in the treatment of hypercholesterolemia. Simvastatin is semi synthetically derived from lovastatin, a secondary metabolite of fungal origin. Bio catalytic production of simvastatin at industrial scale is of great interest because it decreases its production cost well as environmental impact in comparison to chemical synthesis of simvastatin. Simvastatin has been reported as a potential therapeutic agent in the regulation of inflammatory and immune response, bone turnover, neovascularisation, vascular tone, and arterial pressure along with hypocholesteremic property. The present review deals with the structure, biosynthesis, pharmacological properties, therapeutic applications and alternative strategies for biological synthesis of simvastatin.

**Keywords:** Simvastatin, HMG-CoA reductase (HMGR), low density lipoprotein (LDL), high density lipoprotein (HDL), therapeutic applications.

### INTRODUCTION

Hypercholesterolemia is a primary risk factor for the cardiovascular diseases (CVDs), the major cause of death worldwide. Simvastatin, a leading semi synthetic cholesterol lowering drug, was originally developed by Merck under the brand name Zocor®. In 2005, it was Merck's best selling drug and the second-largest selling statin in the world with about \$5 billion in sales. According to IMS health, simvastatin became the most-prescribed statin, with 94 million prescriptions filled in 2010.<sup>1</sup> Simvastatin is an anti-lipidemic prodrug which on in-vivo activation to its  $\beta$ -hydroxy acid (BHA) (Figure 1) acts as an inhibitor of HMGR (HMG Co A reductase).



**Figure 1:** 1. Lactone form 2. Hydroxy acid form of simvastatin

### Compound Information

Name: Simvastatin

Molecular formula:  $C_{25}H_{38}O_5$

Molecular weight: 418.566

IUPAC name: (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl] -ethyl} -3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen -1-yl 2,2-dimethylbutanoate)

The stereochemistry of the side chain ester moiety is not important for inhibitory binding to HMGR, as the spatial requirements of the acyl moiety are compatible with compact, branched-chain aliphatic acyl groups, and additional branching at the  $\alpha$  carbon of the acyl moiety increases potency.<sup>2</sup> An attractive property of statins is that they reduce levels of bad low-density-lipoprotein (LDL), but slightly increase or keep the levels of good high-density lipoprotein (HDL) unaffected.<sup>3</sup>

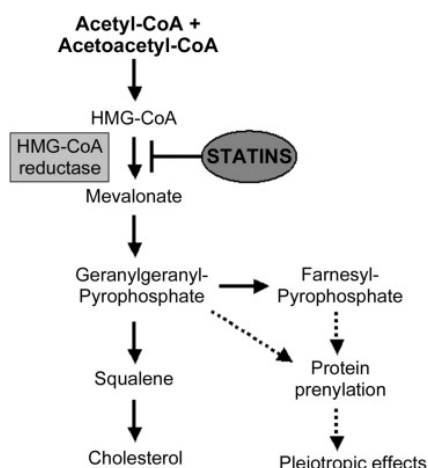
### MECHANISM OF ACTION

Statins in their open hydroxy form (BHA) are competitive antagonists of HMGR as it directly competes with the endogenous substrate (HMG CoA) for the active site cavity of the enzyme (Figure 2). The blocking of HMGR enzyme results in inhibition of cholesterol synthesis via the mevalonate pathway.<sup>2,4-5</sup> The end result is lower LDL (Low Density Lipoprotein), TG (Triglycerides) and total cholesterol levels as well as increased HDL (High Density Lipoprotein) levels in serum.<sup>6-7</sup> Therefore, statins are important drugs for treating cardiovascular diseases (CVDs) and other clinical implications related to higher cholesterol level.

The essential structural components of all statins are dihydroxyheptanoic acid unit and a ring system with different substituent. The statin pharmacophore is modified hydroxyglutaric acid component, which is structurally similar to the endogenous substrate HMG



CoA. This similar configuration results in the binding to the same active site of the enzyme finally inhibiting HMG-R activity.<sup>8</sup> The crystal structures of the catalytic portion of human HMG-R with some substrate and product complexes (HMG-CoA, HMG, CoA, and NADPH) provide a detailed view of the enzyme active site.<sup>9</sup> The statins occupy a portion of the HMG-CoA binding site, thus blocking substrate access to the active site of the enzyme, mediated by a large number of van der Waal interactions between the inhibitors and HMG-CoA reductase.<sup>10</sup>

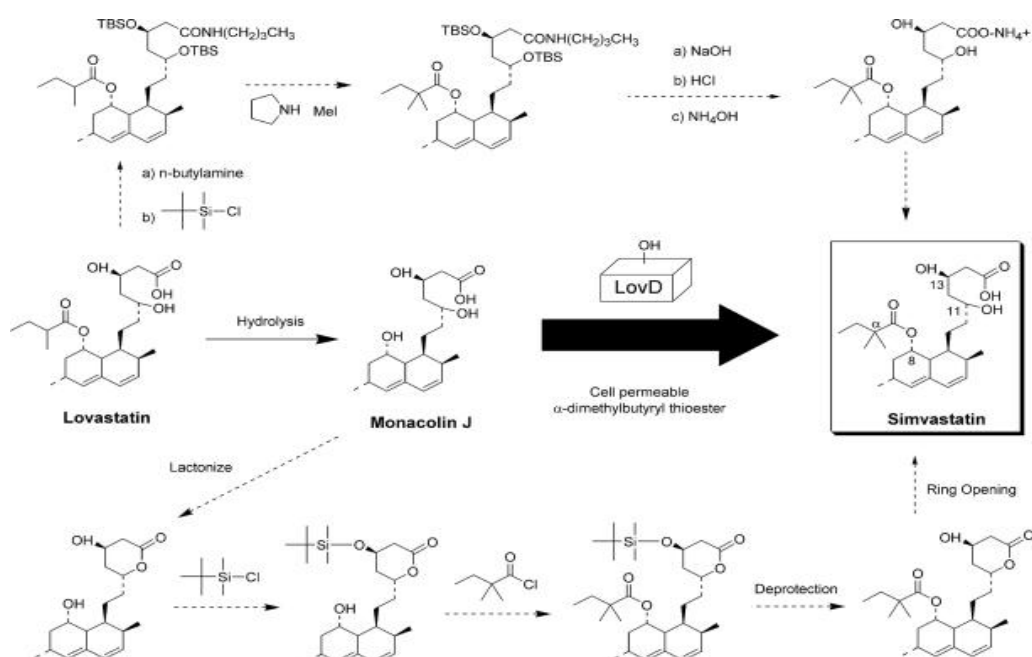


**Figure 2:** Inhibition of cholesterol biosynthesis by statins by blocking the key enzyme HMG-CoA-reductase (HMGR) [5]. (CoA = coenzyme A; HMG = hydroxy-methylglutaryl.)

### BIOLOGICAL PRODUCTION OF SIMVASTATIN

The first statin to be discovered was Mevastatin. It was isolated in 1976 from the fungal source, *Penicillium*

*citrinum* and named ML- 236B.<sup>11-12</sup> At the same time it was also isolated from another strain, *Penicillium brevicompactum* and named compactin.<sup>13-14</sup> However, Lovastatin was the first statin to be approved by the United States Food and Drug Administration (1987) and made available in the pharmaceutical market as an anticholesterolemic drug.<sup>15</sup> Simvastatin is a semi synthetic compound derived from the natural product lovastatin, a fungal polykeide produced by *Aspergillus terreus* as a secondary metabolite.<sup>16-18</sup> The synthesis of simvastatin from lovastatin is a multistep process involving replacement of 2-methylbutyryl side chain with 2, 2-dimethylbutyryl group with an additional methyl group at the C2' position of the lovastatin side chain. Two semi synthetic processes<sup>2,5</sup> are widely used to synthesize simvastatin starting from lovastatin (Figure 3). The most commonly adopted process starts with the hydrolysis of lovastatin to yield the key intermediate monacolin J, followed by the lactonization of the acid to protect the C11 hydroxyl group and trimethylsilylation protection of the C13 hydroxyl. The protected monacolin J is then subjected to acylation by  $\alpha$ -dimethylbutyryl chloride to yield the protected form of simvastatin, which is subsequently deprotected to yield simvastatin.<sup>19-20</sup> Even after considerable optimization, the chemical processes have major disadvantages as 1) overall yield is less than 70 percent, 2) involve mass-intensive multistep reactions, 3) require huge amounts of toxic and hazardous reagents along with harsh reaction conditions, and 4) final product is difficult to purify involving high cost in labour and environment protection.



**Figure 3:** The bio catalytic reaction studied is the enzymatic conversion of monacolin J to simvastatin (thick arrow). LovD is able to regioselective acylate the C<sub>8</sub> hydroxyl group. Two commonly used semisynthetic processes are shown with dashed arrows.<sup>22</sup>

To avoid all these drawbacks and with the growing interest in enzymatic biocatalysis alternate, more environment friendly process have been developed. Advances in the research of lovastatin biosynthesis led to the recognition that simvastatin could be obtained via a selective enzymatic deacylation of Lovastatin.<sup>21</sup> Yang *et al* (2007) identified a biocatalyst Lov D (an acyltransferase) from the lovastatin biosynthetic gene cluster of *Aspergillus terreus* that regio-selectively transfers the methylbutyryl group from the lovastatin diketide synthase to the C8 hydroxyl group of monacolin J to yield simvastatin and described the cloning and characterization of this dedicated acyltransferase.<sup>22</sup>

Lov D has broad substrate specificity towards the acyl carrier, the acyl group, and the decalin core and thus has the ability to catalyze the direct acylation of monacolin J. Moreover, monacolin J is prepared in a single step from inexpensive precursors.<sup>23</sup> Using this methodology, a whole-cell bio catalytic process is able to convert monacolin J to simvastatin in a highly efficient single step procedure, exploiting the properties of Lov D. The fermentation process can be easily scaled up to produce simvastatin at an industrial-scale. The use of this process with approximately 1,000-fold improved enzyme pushed the reaction to completion at high substrate loading; minimizing the amounts of acyl donor as well as of solvents for extraction and product separation and has been used to manufacture over 10 metric tons of simvastatin till date.<sup>24</sup>

### Alternative Hypothesis

Only few fungal strains (mainly *Aspergillus terreus*) have been used for industrial scale production of statin molecules (mainly lovastatin and simvastatin). Finding a novel source of acyltransferase or related class of enzyme with a potential to synthesize HMGR inhibitor molecules will represent an important milestone in biocatalysis of statin or their analogs. "*Bacterial fatty acid synthesis gene clusters are analogs of fungal polyketide synthesis gene clusters so they must possess related enzymes which could be explored as potential biocatalysts for production of statins*".

Based on this hypothesis other microorganisms from natural sources should be screened for a) inherent capability to produce statins as secondary metabolites, b) one step conversion of Monacolin J to Simvastatin in the presence of thioesters in the fermentation media. It could be a lead to potential microbial strains as statin producers which could provide a new industrially important strain for production of statins.

### PHARMACOLOGICAL PROPERTIES

Simvastatin is twice as potent as Lovastatin.<sup>25</sup> It lowers serum cholesterol by inhibiting hepatic synthesis of cholesterol and by increasing the number of low-density lipoprotein (LDL) receptors present on hepatic cellular membranes.<sup>26</sup> Simvastatin, when used at doses of 40 mg/day in patients with heterozygous familial

hypercholesterolemia, significantly reduces total cholesterol (>30 percent) and LDL cholesterol (35-45 percent) and tends to reduce triglycerides with raised high-density lipoprotein (HDL). The reductions from baseline were approximately 20 to 40% for serum levels of total cholesterol, 35 to 45% for low density lipoprotein (LDL)-cholesterol and 10 to 20% for triglycerides in patients with primary hypercholesterolemia. In general, simvastatin was also more effective than standard dosages of bile acid sequestrants, fibrates or probucol in lowering serum levels of total cholesterol and LDL.<sup>27,28</sup>

Simvastatin even though absorbed well from the gastrointestinal tract, is highly extracted by the liver and only 7% of the administered dose reaches the general circulation. The peak inhibition of HMG-CoA reductase activity occurs within 2 to 4 hours of drug intake. Increasing the dose of Simvastatin from 5 to 120 mg increases the pharmacological activity in a linear fashion. Several metabolites tend to remain within the liver and intestine and reabsorption of some metabolites may occur. Simvastatin is eliminated mainly in the faeces due to biliary excretion but only a small percentage of the dose is found in the stool in the form of the parent compound or simvastatin acid.<sup>29</sup>

Comparative studies with other HMG-CoA reductase inhibitors (lovastatin, pravastatin and fluvastatin) demonstrate greater reductions in serum levels of total cholesterol and LDL-cholesterol with simvastatin than equal dosages of lovastatin or pravastatin. The greatest LDL reduction is obtained with atorvastatin and simvastatin among all the statins.<sup>30</sup> Reductions in serum levels of total cholesterol and LDL-cholesterol were similar between agents only when lovastatin or pravastatin were administered at a total daily dosage twice that of simvastatin and when fluvastatin was administered at a total daily dosage approximately 8 times that of simvastatin.<sup>28</sup> Thus simvastatin is one of the best drugs available to treat the hypercholesterolemia related problems.<sup>31</sup>

### THERAPEUTICAL APPLICATIONS

Simvastatin has a number of pleiotropic effects. In the last few years, many studies have demonstrated that in addition to their lipid lowering effects, statins have anti inflammatory and immunomodulatory properties, including reducing the perioperative risk of cardiac complications and sepsis. This has led to the suggestion that 'at risk' patients should be treated with statins before operation. Recent studies have suggested that statins could have beneficial effects in immune mediated neurological disorders like Alzheimer's disease (AD) and Parkinson's disease. All the clinical applications of simvastatin are summarized below in table 2:



**Table 2:** Therapeutic applications of Statins

S.No	Disease	Possible underlying mechanism	Reference
1	Cholesterol Lowering	Inhibits the mevalonate pathway by blocking the rate limiting step of pathway via inhibiting HMGR	[4]
2	Cardiovascular Disease (CVD)	Plaque stabilization, improvements in endothelial-mediated responses with better local regulation of the coronary arterial tone and an immunosuppressive effect.	[32-34]
3	Anti Inflammatory & Anti oxidant	Reduce the plasma levels of inflammatory markers like CRP due to an inhibition of IL-6 in the vascular tissues. Inhibit the ability of macrophages to oxidise LDL.	[35]
4	Bone Regeneration	Promote osteoblastic & inhibit osteoclastic activity.	[36]
5	Cancer	Simvastatin on LNCaP and PC3 cells showed its ability to inhibit serum-stimulated Akt activity and reduced expression of PSA. Akin to this, inhibited serum-induced cell migration, invasion, colony formation, and proliferation.	[37]
6	Alzheimer's Disease (AD)	Modulation of amyloid-precursor protein (APP) cleavage by altering membrane cholesterol levels in vitro. It completely rescued cerebrovascular reactivity, basal endothelial nitric oxide synthesis, and activity-induced neuro metabolic and neurovascular coupling.	[38-39]
7	Parkinson's Disease	Simvastatin exposure inhibited the activation of p21ras (necessary for the neurotoxic chemical to produce Parkinson's) in the microglial cells. The statin also blocked the neurotoxin from activating nuclear factor-kappa B, "a transcription factor required for the transcription of most of the pro inflammatory molecules.	[40]
8	Infectious Diseases	Improved susceptibility to endothelial nitric oxide synthase stimulation and reduced endothelial adhesion of leukocytes, results in improved survival after sepsis.	[41]
9	Renal Diseases	Slow progression of Chronic Kidney Diseases by improving the lipid profile as well as by affecting inflammatory cell-signaling pathways that control vascular cell migration, proliferation, and differentiation.	[42]
10	Acute Lung Injury (ALI)	Vascular-protective changes in EC phenotype can be attributed to statin-induced inhibition of mevalonate production and the resultant changes in Rho GTPase activity and localization	[43]
11	Rheumatoid Arthritis (RA)	Adjuvant therapy associated with other conventional therapeutic methods used in RA. Statins improves endothelial function in patients with RA. Its beneficial effect may be attributed to lowering pro-inflammatory CRP and TNF-alpha concentrations.	[44]
12	Aging	By telomerase activation, statins may represent a new molecular switch able to slow down senescent cells in tissues and be able to lead healthy lifespan extension.	[45]
13	Cognitive disorders	Due to their cholesterol-lowering effects, increase the soluble RAGE level by inducing RAGE (receptor for advanced glycation end products ) shedding, and by this way might prevent the development of RAGE-mediated pathogenesis.	[46]
14	Pancreatic Diseases	Use of statin therapy was associated with a lower risk of pancreatitis in patients with normal or mildly elevated triglyceride levels	[47]
15	Pregnancy Complications	Prevent preeclampsia by decreased release of the anti-angiogenic molecule sFlt-1 from macrophages & increased release of VEGF and PlGF to restore angiogenic balance.	[48]
16	Healing Disorders	Decreasing farnesyl pyrophosphate, facilitating vascular relaxation, promoting neovascularization and reducing bacterial load	[49]
17.	Multiple sclerosis	Attributed to the immunomodulatory properties of statins and to their induction of a bias toward Th2 cell antiinflammatory cytokine production	[50]

## CONCLUSION

Simvastatin inhibits HMG-CoA reductase competitively; decreases LDL level more than other cholesterol-lowering drugs and lower triglycerides level in hypertriglyceridemic patients. Mevalonate metabolism creates a series of vital isoprenoids for different cellular functions, ranging from cholesterol synthesis to the control of cell growth and differentiation, thus HMG-CoA reductase inhibition has

beneficial pleiotropic effects. Simvastatin has become the therapy of choice for the treatment of many dyslipidaemias in the patients, Alzheimer's disease, cancer treatment, bone fracture treatment and used as a general anti-inflammatory agent. Although simvastatin shares a common mechanism of action with other statins, but it shows relatively better efficacy for improving the lipid profile justifying its use as a safe and effective drug



in clinical practice. Hence finding a new source of statins will represent an important milestone in biosynthesis of HMG-R inhibitors and their derivatives and successful engineering of a biosynthetic enzyme into an industrially useful manufacturing tool would lead to substantial cost reduction and potentially novel derivatives for therapeutic applications.

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