From fungus to pharmaceuticals – the chemistry of statins

P.A.C. McPherson*

Belfast Metropolitan College, School of Applied Science (Chemistry), Belfast, UK

Abstract: It has long been recognised that high circulating levels of cholesterol are associated with the development of cardiovascular disease. With the discovery of the cholesterol biosynthetic pathway in 1950, it was soon realised that blockade of key conversions in this pathway may provide useful therapeutic targets for the management of hypercholesterolaemia. In the 1970s the first useful inhibitors of cholesterol biosynthesis were isolated, and paved the way for what would become a multimillion dollar pharmaceutical industry. Modern-day statins are incredibly effective hypolipidaemic agents, interrupting cholesterol biosynthesis at the rate-limiting step through a competitive inhibition mechanism. These compounds' structures interact with key amino acid residues through a variety of defined bonding interactions, and by understanding how these interactions form, better, and safer, hypolipidaemic agents were found. This review describes the historical development of statins and brings us up-to-date with current structure-activity relationships between statins and their target enzyme.

Keywords: Cardiovascular disease, Cholesterol, HMG-CoA reductase, Hypercholesterolaemia, Lipoproteins.

INTRODUCTION

In the early 1900s, Russian pathologist Nickoli Anitschlkow was the first to make the connection between high levels of blood cholesterol and the development of fatty, atherosclerotic plaques – the prelude to cardiovascular diseases such as heart attack and stroke. With the elucidation of the cholesterol biosynthetic pathway some fifty years later, it soon became obvious to scientists that inhibition of this pathway could provide a useful (and lucrative) therapeutic target. What followed was almost forty years of intensive research and development into the class of hypolipidaemic drugs we now call statins. There are a number of excellent reviews detailing the clinical utility of statins in the treatment of hyperlipidaemias [1-3]. However, few have drawn together the different strands in the history of statins, bringing to the reader's recollection the number of individual research groups involved in the development of these life-changing drugs. Therefore, in this current article, we will review the key stages in the development of statins, highlighting areas in their medicinal chemistry and considering the way ahead for these remarkable drugs.

DYSLIPIDAEMIA & CARDIOVASCULAR DISEASE

The connection between 'fat' (cholesterol and triglycerides) and cardiovascular disease originated with the work of German pathologist Virchow, who reported observing a yellow, fatty substance (cholesterol) in the artery walls of cadavers who had died from occlusive vascular disease. He named this condition atheroma (Latin, '*atheroma*', meaning grul-like puss; from the original Greek, '*athera*', grul), but few of his contemporaries believed that there was a connection between atheroma and heart disease [4]. It wasn't until the landmark Framingham study in the 1950s that a firm link between high levels of cholesterol and increased risk of cardiovascular disease was made [5]. This marked the establishment of the lipid hypothesis of cardiovascular disease, which although still refuted by some [6], remains the consensus opinion of the scientific community, and has been responsible for massive body of scientific output over the past five decades.

THE SYNTHESIS & STRUCTURE OF CHOLESTEROL

Cholesterol (5-cholesten-3β-ol) is one of the most widely occurring steroids in mammals, and was first isolated from gallstones by François Poulletier de la Salle in 1769 [7]. Within its structure (Fig. **1**), cholesterol contains eight tetrahedral stereocentres, yielding an impressive 2^8 (or 256) potential stereoisomeric forms. This lead to some confusion in the early 1920s, when German chemists Aldolf Windaus and Heinrich Wieland proposed the first hypothetical structure for cholesterol. Although their initial attempt was later proven as incorrect, subsequent co-operation with British scientists John Bernal and Dorothy Hodgkin lead to the correct structure determination in 1937 [8].

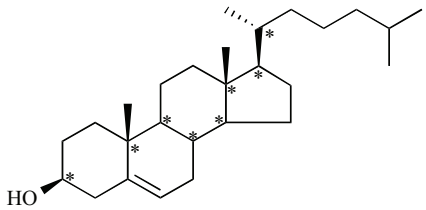


Fig. (1). Stereochemistry of cholesterol. The eight stereocentres denoted by an asterisk (*); the double bond is in the (*Z*)-configuration.

*Address correspondence to this author at the Belfast Metropolitan College, School of Applied Science (Chemistry), Belfast, BT6 9DJ, UK; Tel: 00 44 2890 265000; Fax: 00 44 2890 265001;
E-mail: pmcpherson@belfastmet.ac.uk

1875-5607/12 $58.00+.00

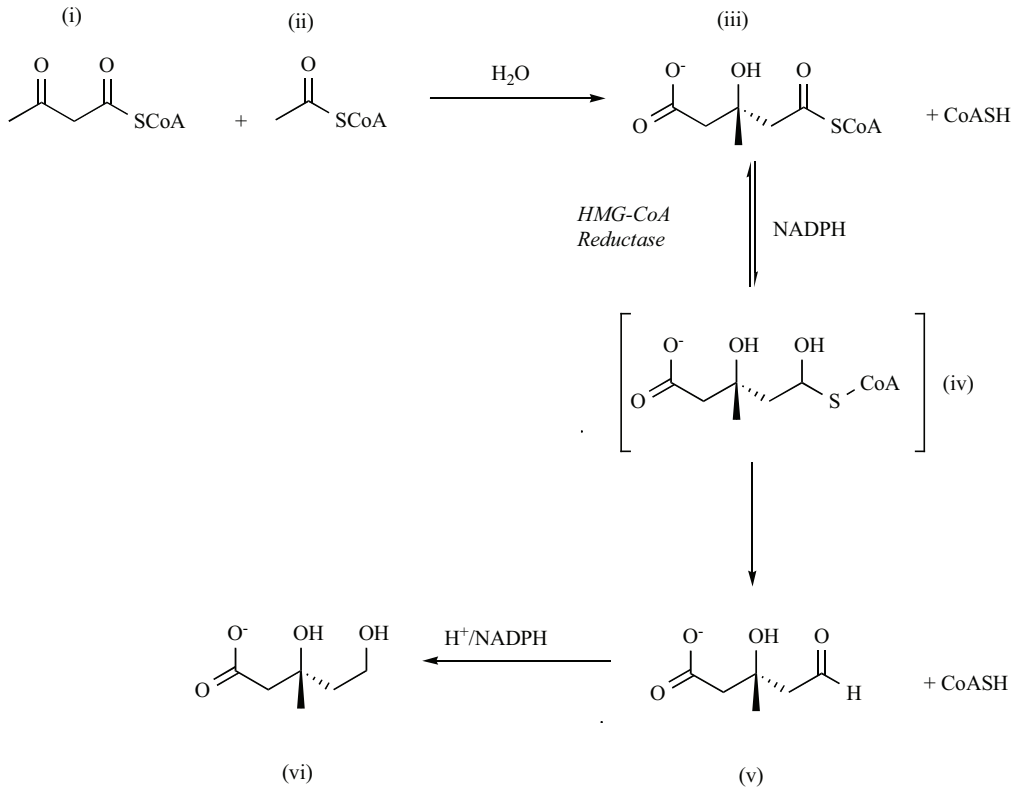


Fig. (2). Rate-limiting step of cholesterol biosynthesis and the formation of (*R*)-mevalonate. This pathway is initiated by an aldol condensation between (**i**) and (**ii**) forming HMG-CoA (**iii**), which is initially reduced to a mevaldyl-CoA hemi-thioacetal intermediate (**iv**) and then decomposes to mevaldehyde (**v**). A final reduction produces (*R*)-(+)-mevalonate (**vi**).

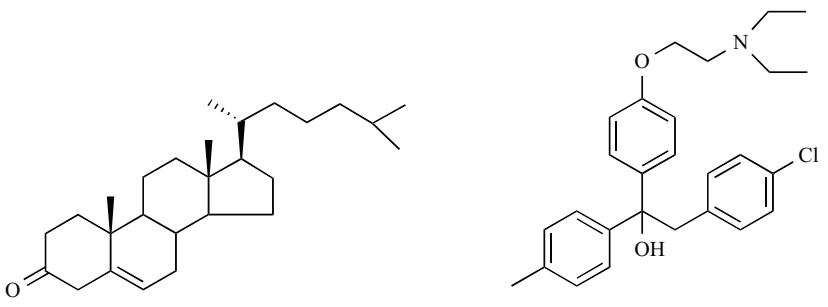
Approximately 10 years later, Konrad Bloch, working at the University of Harvard, demonstrated that cholesterol was synthesised *de novo* from acetyl-CoA, a key intermediate in mammalian metabolism [9]. Over the ensuing years, the cholesterol biosynthetic pathway evolved into the form familiar to many biochemistry undergraduates. In this pathway, acetyl-CoA and acetoacteyl-CoA condense to form the six-carbon compound β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). This compound becomes bound to the cytosolic enzyme HMG-CoA reductase – a tetramer with two active sites, one for HMG-CoA and another for NADPH. The thiol-ester group of HMG-CoA is initially reduced to mevaldyl-CoA (using NADPH as reducing power), which then undergoes acid-catalysed conversion to mevaldehyde and coenzyme A (CoA-SH), before finally becoming reduced to a primary alcohol (mevalonic acid; Fig. (**2**)). This four-electron reduction represents the rate-limiting step of cholesterol biosynthesis, and a series of subsequent phosphorylations, decarboxylations and an isomerisation finally produces cholesterol [10].

TRANSPORT OF CHOLESTEROL

Despite its much maligned nature, cholesterol plays a vital role in mammalian physiology; it is used in the synthesis of cell membranes and a variety of steroid hormones, including aldosterone, cortisol, testosterone and 17β-oestradiol [11]. In mammals, cholesterol is derived from two sources – *de novo* synthesis *via* the isoprenoid pathway and the diet. Dietary cholesterol is mainly derived from animal fats, which are packaged into lipid-protein complexes known as chylomicrons [12]. These lipoproteins, which are part of the exogenous pathway of lipoprotein metabolism, are acted on by a variety of enzymes, producing remnant chylomicrons, which are taken-up by hepatic chylomicron receptors. The lipids are repackaged and released from the liver as very low density lipoproteins (VLDL), which undergo remodelling to low density lipoproteins (LDL). These latter particles are a cholesterol-rich species, and transport cholesterol to extra-hepatic tissues for biosynthetic processes [13].

Approximately 75 % of LDL is removed from the circulation by the hepatic LDL-receptor, with the cholesterol content ultimately being conjugated for excretion as bile acids (*ca*. 500 mg per day)[1] [14]. LDL can undergo oxidative modification by a number of free radical species, which impairs its uptake by the LDL-receptor [15]. Thus, LDL is often regarded as a better indicator of cardiovascular risk than cholesterol alone. Of particular relevance to this discussion is cholesterol's role in regulating the synthesis of HMG-CoA reductase; cholesterol inhibits the transcription of the gene encoding this enzyme, effectively stopping *de novo* cholesterol synthesis when intracellular cholesterol levels are high [16]. Excess cholesterol is recovered from

[1]Bile acid sequestrants (*e.g.* cholestyramine) are effective hypolipidaemic drugs which bind bile acids, preventing their re-absorption and promote hepatic removal of LDL through a concomitant up-regulation of the LDL-receptor. However, these drugs are frequently associated with gastrointestinal side-effects.



Δ^4-Cholestenone Triparanol

Fig. (3). Early hypolipidaemic drugs, Δ^4-cholestenone and Triparanol.

extra-hepatic cells by high density lipoproteins (HDL), in a process termed 'reverse cholesterol transport' [17]. This pathway represents the major physiological means of reducing extra-hepatic cholesterol levels, and manipulation of this pathway by pharmacological agents is the subject of recent research [18].

EARLY INHIBITION OF CHOLESTEROL SYNTHESIS

The initial attempts to reduce *de novo* cholesterol synthesis were plagued with problems. Cholesterol synthesis was shown to be inhibited by Δ^4-cholestenone; however, it was soon revealed that this compound lead to accumulation of dehydrocholesterol in mammals, which in itself accelerated the growth of fatty streaks [19]. Shortly thereafter, Blohm and co-workers reported the discovery of a new inhibitor of cholesterol synthesis which prevented the reduction of desmosterol to cholesterol. This drug, marketed commercially as Triparanol (Fig. **3**), was once again associated with major side-effects, prompting its withdrawal from the market in the 1960s [20]. Following these early failed attempts, it became obvious that inhibition of the rate-limiting step of cholesterol synthesis would be a logical target for drug design. Having identified the enzyme responsible for the rate-limiting step in cholesterol biosynthesis some years earlier, the next stage in the journey towards the development of statins was to identify HMG-CoA reductase inhibitors which would have minimal side-effects.

DISCOVERY OF THE FIRST STATIN

In 1971, Akira Endo and Masao Kuroda started work on a project searching for microbe-derived compounds which would block the isoprenoid pathway in other microbes, and thus act as antibiotic drugs. Over the ensuing two years, they screened over 6000 microbes, testing each for the ability to block cholesterol synthesis. Their first breakthrough came from the isolation of the antibiotic citrinin from the mould

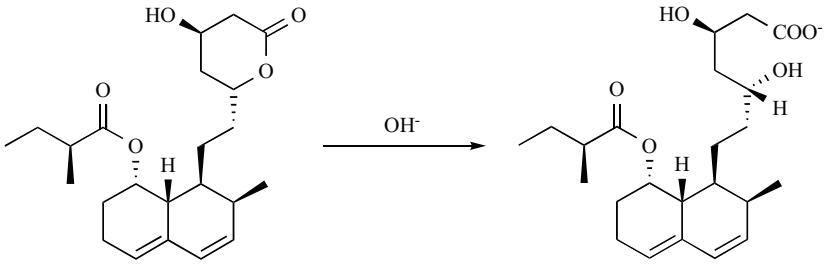
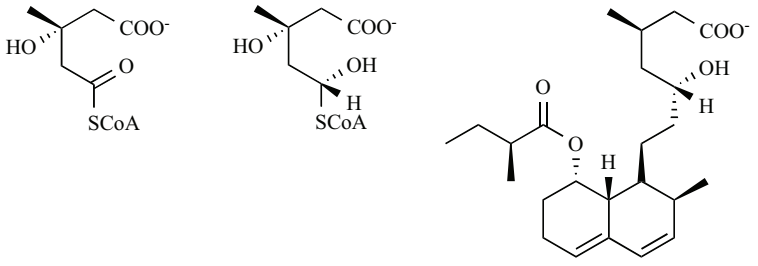


Fig. (4). Alkaline hydrolysis of the prodrug form of mevastatin to the active open-acid form. This reaction is mediated *in vivo* by esterases.



HMG-CoA Mevaldyl-CoA Mevastatin

Fig. (5). Structural homology between HMG-CoA, mevaldyl-CoA and mevastatin (open-acid form).

Pythium ultimum. Citrinin was shown to be a potent inhibitor of HMG-CoA reductase, and subsequent experiments with cultures of *Penicillium citrinum* lead to the isolation of mevastatin – the first statin [21]. The analogue of HMG-CoA in metastain was found to be a lactone (cyclic ester), which is hydrolysed *in vivo* by esterases, forming a dihydroxyheptanoic acid group (Fig**. 4**). Since it was found that the open-acid form of the drug was a much more potent hypolipidaemic agent, this implied that the structurally similar dihydroxyheptanoic acid group must bind to the enzyme's active site [22]. Later structural determinations revealed that although mevastatin is similar to HMG-CoA, it is actually more similar to mevaldyl-CoA (Fig. **5**). This suggested that statins, such as mevastatin, may actually mimic the transition-state intermediates in HMG-CoA reduction, rather than the substrate [23].

In 1979, mevastatin (marketed as Compactin) was trailed in patients with severe hypercholesterolaemia in over ten centres across Japan. Despite its success in the laboratory, mevastatin did not progress beyond these clinical trials, as simultaneous animal work identified significant cumulative toxicity [24]. However, Endo and co-workers had successfully identified the key features of the HMG-CoA reductase inhibitor pharmacophore and provided a framework for the development of further inhibitors of *de novo* cholesterol synthesis. During the mevastatin trials, scientists at Merck reverse synthesised mevastatin and subsequently produced lovastatin, a structurally-similar molecule with an additional C^3-methyl group on the hexahydronapthylene ring structure. Clinical trials of lovastatin were a resounding success and lead to the wide scale release of the drug in 1987, thus making lovastain the first clinically useful statin [25].

MODERN-DAY STATINS

Medicinal Chemistry

Following the success of lovastatin, others quickly followed. Simvastatin, released in 1988, was the first semi-synthetic statin, produced by methylation of lovastatin's methylbutyrate ester, and pravastatin, produced by biotransformation of mevastatin, was released in 1991 [26]. Strictly speaking, lovastatin and simvastatin are prodrugs – they are manufactured with an intact lactone structure in the head group, which is then hydrolysed *in vivo* by esterases to produce the polar, acyclic head characteristic of substrates for HMG-CoA reductase. Lovastatin, simvastatin and pravastatin are collectively referred to as 'type I', or 'first generation' statins (Table **1**), and are typified by a partially reduced naphthylene ring system, with one or two methyl substituents, and a methylbutyrate ester. The α-methyl group at position C^6 on the reduced naphthylene ring significantly enhances HMG-CoA reductase inhibition – *e.g.* IC_{50} lovastatin (C^6-CH_3 substituted) 24 nM *vs*. pravastatin (C^6-OH substituted) 1900 nM [27]. Although the type I statins are still used in clinical settings, they tend to be less well tolerated and have a relatively low bioavailability [28]. Furthermore, the large number of stereocentres within type I statins' structure makes them difficult to synthesise artificially [29], and the commercially marketed drugs are supplied as racemates, even though HMG-CoA reductase only binds the 3*R*, 5*R* isomer of statins.

Second generation (type II) statins were first made available in 1994, with fluvastatin paving the way for these newer, more efficacious drugs [30]. The type II statins are characterised by a larger hydrophobic nucleus, free from potentially problematic stereocentres. The chemical nature of this hydrophobic nucleus varies – pyrrole (atorvastatin), indole (fluvastatin), pyrimidine (rosuvastatin), pyridine (cerivastatin) and quinoline (pitavastatin) – and is substituted by at least two further lipophilic groups: a fluorophenyl group (all statins) and an isopropyl group (all except pitavastatin). It is well understood [31] that the relative lipophilicity of statins controls their pharmacological activity and thus the greater the lipophilicity, the lower the hepatoselectivity [32]. Therefore, on comparison with the first generation (hydrophobic) statins, the more hydrophilic statins (*e.g.* pravastatin and rosuvastatin) tend to be highly hepatoselective. However, this can also lead to hepatotoxicity at moderate concentrations, as these drugs can accumulate in hepatocytes [33].

Pharmacodynamics & Safety of Statins

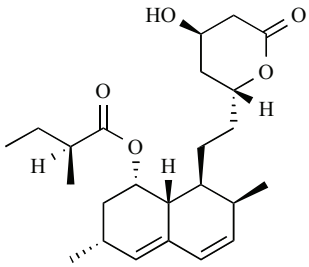
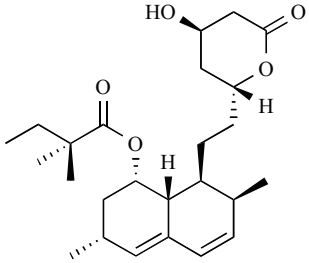
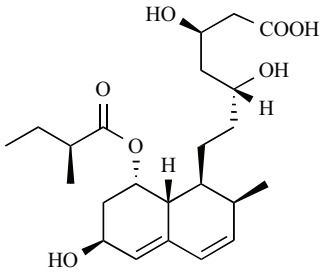
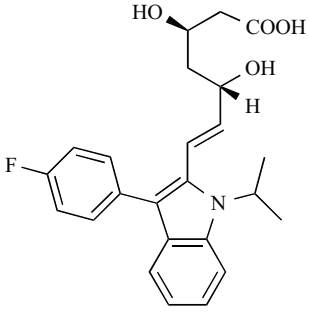
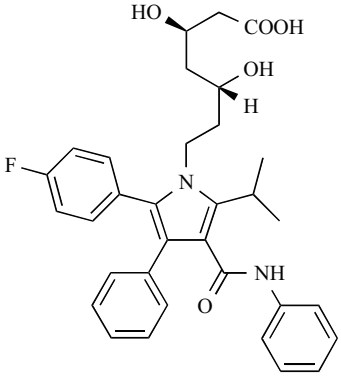
All statins are absorbed quickly following their administration and reach a peak plasma concentration within four hours. The majority of statins exhibit a low systemic bioavailability, suggesting extensive first-pass removal by the liver [34]. However, given that the liver is the target organ for statins, the substantial first-pass removal could be beneficial. The metabolism of statins in mediated predominately by the cytochrome P450 system, after which the majority of the metabolites are eliminated *via* the bile duct [35]. The metabolism of statins by the P450 system poses a problem when the administration of the drug coincides with consumption of grapefruit; furanocoumarins, *e.g.* bergamottin, inhibit one of the P450 isoforms (CYP3A4) and substantially reduce the metabolism of statins. As a consequence, the clearance of the active drug is greatly reduced, increasing the risk of hepatotoxicity [36]. In this regard, pravastatin and rosuvastatin would appear to be the safest of the statins, as they are not metabolised by the CYP3A4 system, and are excreted *via* the renal system in a largely unmodified form [37].

Although statins are generally well tolerated, a few serious side effects have been reported [38], mainly as fatal or nonfatal rhabdomyolysis. The withdrawal of cerivastatin in 2001 was due to high incidence of rhabdomyolysis, although such a high degree of mortality and morbidity appears to be specific to this drug [39]. In general, the incidence of myopathy associated with statins occurs at a frequency of *ca*. 1/1000 cases, and would seem to be dose-related [40]. The incidence of rhabdomyolysis is a much rarer event (*ca*. 1/10000 cases), and overall, the toxicity reported for statins would appear to be minimal, with the FDA regarding these drugs as having a very favourable risk-to-risk ratio [41].

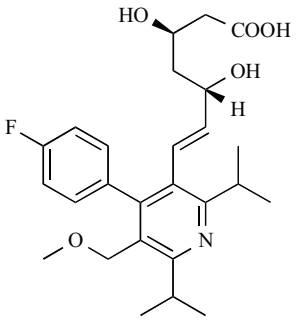
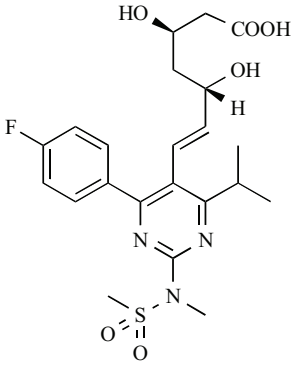
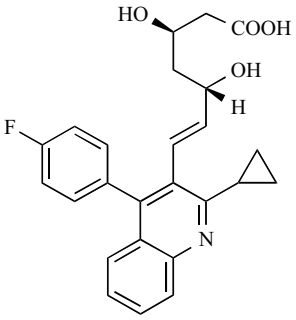
Mechanism of Action

The basic principle of statins' mechanism of action is that they mimic HMG-CoA and occupy the active site of HMG-CoA reductase in a competitive fashion, without undergoing enzymic reduction. The early work of Endo *et al*. identified

Table 1. Summary of the Physicochemical Properties and Clinical Effectiveness of Statins. Pharmacokinetic Data Taken from Reference [63]

	Drug Name (*Proprietary name*)	Structure	Year Released	IC_{50} (nM)	Bioavailability	Serum LDL Reduction[a]
Class I Statins	Lovastatin *(Mevacor, Altocor & Altoprev)*		1987	24.0	5 %	34 %
	Simvastatin *(Zocor & Lipex)*		1988	22.0	5 %	41 %
	Pravastatin *(Pravachol, Selektine & Lipostat)*		1991	1900.0	18 %	34 %
Class II Statins	Fluvastatin *(Lescol)*		1994	28.0	24 %	24 %
	Atorvastatin *(Lipitor & Torvast)*		1997	8.0	12 %	50 %

(Table 1). Contd…..

	Drug Name (Proprietary name)	Structure	Year Released	IC50 (nM)	Bioavailability	Serum LDL Reductiona
Class II Statins	Cerivastatin *(Lipobay & Baycol)*		1998	10.0	60%	28 %
	Rosuvastatin *(Crestor)*		2003	5.0	20 %	63 %
	Pitavastatin *(Livalo & Pitava)*		2009	6.8	80 %	48 %

[a]Data obtained for reduction in LDL-cholesterol in hypercholesterolaemic patients prescribed 40 mg (o.d.) atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin and rosuvastatin; 4 mg pitavastatin and 0.3 mg cerivastatin [64-66].

the key features of a successful statin, but at that stage, the crystal structure of the catalytic domain of HMG-CoA reductase had not been solved. However, the work of Istavan *et al*. [42] led to the crystal structure of HMG-CoA reductase being solved in 2000, which provided insight into the catalytic mechanism of the enzyme. With regard to its natural substrate, HMG-CoA reductase utilises nine main residues to bind HMG-CoA (Fig. **6**); the main interactions are:

1. Lys^{691} found in the *cis*-loop domain of the enzyme, forms an ion-dipole bond with the carbonyl oxygen at C^1 in HMG-CoA, and therefore promotes the reduction of the carbonyl oxygen to a primary alcohol during catalysis.
2. Asp^{690} and Ser^{684} work in unison to stabilise the C^3-hydroxy group through hydrogen bonding (serine) and ion-dipole bonding (aspartic acid).
3. Lys^{735} anchors the substrate in the active site through ion-dipole bonding with the substrate's C^5-carboxylate group.

During the normal catalytic process, the CoA chain is directed along a hydrophobic binding pocket through van der Waals interaction with Tyr^{479}. Then, during the first NADPH-mediated reduction, the imidazole nitrogen of His^{866} acid-catalyses the hydrolysis of the thioester bond, liberating CoASH and mevaldehyde.

The mechanism by which statins prevent binding of HMG-CoA to the reductase enzyme was later described by Istavan & Deisenhofer [43]. They found that statins extend into the narrow pocket where HMG normally binds, bending at the O^5-hydroxyl group of the HMG-like moiety, and causing the formation of a shallow furrow which accommodates the hydrophobic ring structure of the statins. The ability of statins to reshape HMG-CoA reductase is due

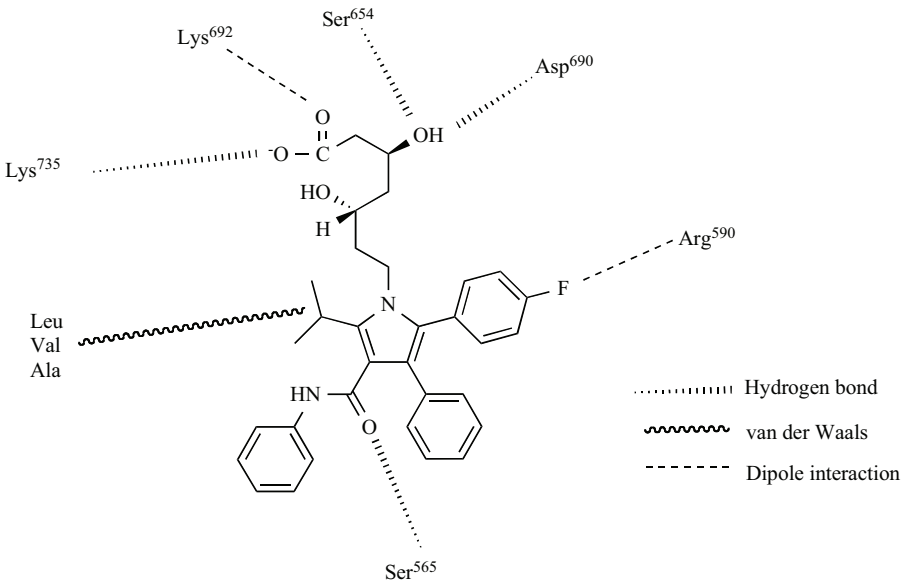


Fig. (6). Binding of atorvastatin to HMG-CoA reductase.

to the intrinsic flexibility of the C-terminal portion of the enzyme, and statins themselves modulate their conformation to maximise contact with this hydrophobic pocket. Rosuvastatin has the greatest number of bonding interactions with the enzyme, which is reflected in its IC_{50} of 5 nM, and is the only statin to bond to Arg^{568}, mediated by the drug's sulfon group. The defining feature of type II statins, the fluorophenyl group, layers on top of the guanidinium group of Arg^{590}, supported by polar interactions between the ε-amino groups of arginine and the fluorine atom. Thus, although it is the dihydroxyheptanoic acid group which binds to the active site, it is the ring structure which predominately dictates the binding affinity of the inhibitor and ensures that the drug is not displaced by the enzyme's natural substrate.

SYNTHESIS OF STATINS

One of the challenges the pharmaceutical industry now faces is devising new synthetic routes for the preparation of drugs, especially in light of the current move towards 'green chemistry' techniques. For the type I statins, this is less of a concern, as they have biological origins and can be produced by batch fermentation methods [44]. It would therefore seem possible that the skeleton of a statin can be produced biologically [45]; however, functional group substitution of this skeleton can generally only be achieved through chemical modification. Thus, in the synthesis of simvastatin, biologically-produced lovastatin is first hydrolysed to monacolin J, which then undergoes lactonization, silylation, acylation, deprotection and ring opening to form the product (Fig. **7**) [46]. Despite optimisation, this route still produces a yield under seventy-percent, and has an unfavourable atom economy due to the number of protection/deprotection steps.

A potential 'green' synthetic route for the production of simvastatin utilises a recombinant acyltransferase, LovD, usually expressed in *Aspergillus terreus*, which in the presence of α-dimethylbutyryl-*S*-methyl mercaptopropionate, transfers acyl groups to lovastatin, producing simvastatin [47]. A further potentially useful development for statin medicinal chemistry was the discovery of a deoxyerythronolide B synthase which catalyses a Diels-Alder reaction, producing a lactone moiety [48].

The synthesis of type II statins is even more heavily reliant on traditional organic chemistry. For example, the industrial production of fluvastatin is based on a scaled-up version of the research synthesis, in which an aldol-like condensation between *E*-[3-(4-fluorophenyl)-1-(1-methyl-ethyl)-1H-indol-2-yl]-2-propenal and *tert*-butyl acetoacetate produces the basic fluvastatin pharmacophore (Fig. **8**) [49]. This process has recently been improved by Novartis, in which a 'one-pot' synthesis increases the overall yield by 25 % and reduces the number of solvents required [50]. A similar, 'one-pot' synthesis for rosuvastatin has been reported by Lek Pharmaceuticals (part of the Sandoz company), in which a Wittig-type reaction is used to couple (2*S*,4*R*)-4-(*tert*-butyldimethylsilyloxy)-6-oxotetrahydro-2H-pyran-2-carbaldehyde with a heterocyclic pyrimidine. This route utilizes a synthetic β-substituted δ-lactone, which is efficiently synthesised in a green, enzymic approach [51-52].

Despite the more complicated side-chains, type II statins do have the potential for biological production methods. The manufacture of atorvastatin utilizes (*R*)-4-cyano-3-hydroxy-butyrate as a key intermediate, and recent research has identified a biocatalytic route for the preparation of this intermediate utilizing 4-chloroacetoacetate, a ketroreductase and a halohydrin dehalogenase [53]. The (*R*)-4-cyano-3-hydroxybutyrate so-formed then undergoes Claisen condensation, borane reduction, protection and hydrogenation to produce the calcium salt of atorvastatin [54].

CONCLUSION AND THE WAY AHEAD – TYPE III STATINS?

Statins are incredibly effective hypolipidaemic agents and have, in the main, been consistently shown to reduce morbidity and mortality in patients with and without cardiovascular disease. Despite the withdrawal of

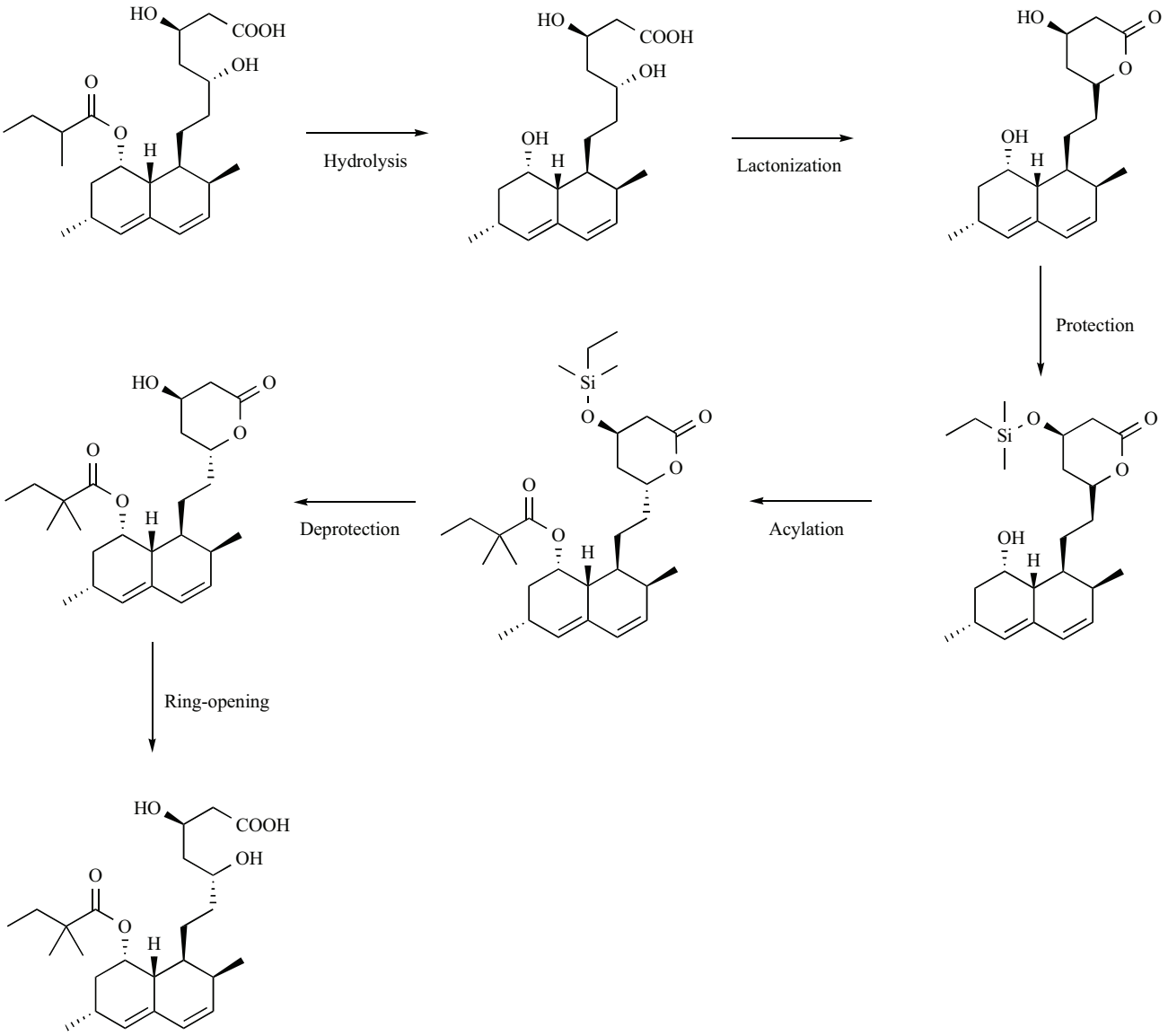


Fig. (7). Synthesis of a type I statin: conversion of lovastatin to simvastatin *via* monacolin J.

cerivastatin in 2001, we are told some statins may become available as over-the-counter (non-prescription) medications, for use in primary prevention initiatives. It should be noted that statins' favourable effects are not limited to reduction of LDL-cholesterol. Various investigations have shown that statins inhibit small guanosine triphosphate–binding proteins and restore nitric oxide levels in the vascular endothelium [55]. The *o*-hydroxy metabolite of atorvastatin has been shown to reduce oxidation of LDL in a dose-dependent fashion [56], and to inhibit formation of F_2-isoprostanes [57]. Such 'pleiotropic' effects of raise the possibility of applying these drugs to other clinical conditions associated with oxidative stress (*e.g.* Alzheimer's disease), and can only serve to encourage their utilization.

In terms of future developments in statins' medicinal chemistry, the impetus will presumably focus on improving synthetic routes and reducing the overall cost of the manufacturing process. Although it is tempting to speculate that the characterisation of HMG-CoA reductase's catalytic domains could lead to type III statins, there are (currently) no new patents. Medicinal chemists at Bristol-Myers Squibb have reported a 'next generation' statin (BMS-644950; Fig. (**9**)) [58], however this has not (as yet) progressed into clinical trials. Similarly, although three-dimensional quantitative structure-activity relationships (3D-QSAR) have identified eight new statin pharmacophores [59], it will be quite some time (if ever) that these findings will produce clinically useful products. The future of hypolipidaemic drugs will most likely arise from inhibition of cholesteryl ester transfer protein (CETP), improving reverse cholesterol transport [60]. Although the early clinical trials of these drugs (specifically torcetrapib) were stopped due to unacceptable mortality [61], recent trials appear more promising [62].

Taking into account that statins were once considered orphan drugs, these fungus-derived compounds have been responsible for billions of dollars of revenue and considerable scientific output. The past fifty years has

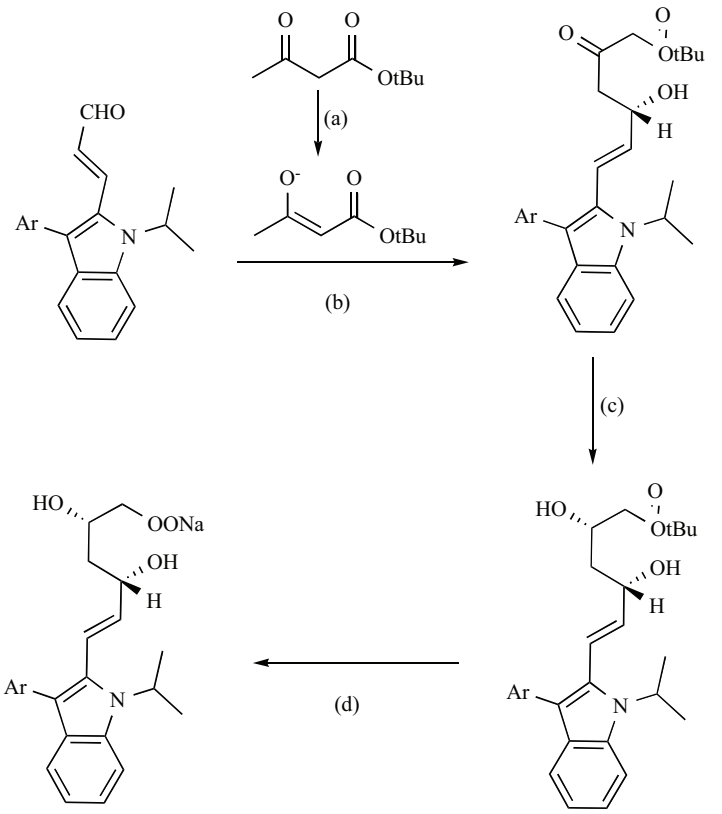


Fig. (8). Synthesis of a type II statin: industrial synthesis of fluvastatin from *E*-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-2-propenal. The main solvents/conditions are: (**a**) THF, 20 – 25 °C; (**b**) THF, 20 – 25 °C; (**c**) THF/MeOH – 78 °C followed by EtOHAc, 20 – 25 °C; (**d**) EtOH, 20 – 25 °C.

provided incredible insight into the biochemistry of cholesterol synthesis, and continued research and development in this field will surely improve the clinical outcome of countless individuals worldwide.

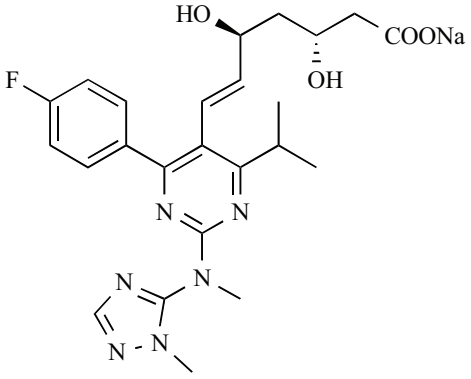


Fig. (9). Chemical structure of BMS-644950, a candidate third generation statin.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Mihos, C.G.; Salas, M.J.; Santana, O. The pleiotropic effects of the hydroxy-methyl-glutaryl-CoA reductase inhibitors in cardiovascular disease: a comprehensive review. *Cardiol Rev.,* **2010**, *18*, 298-304.

[2] Toth, P.P. Drug treatment of hyperlipidaemia: a guide to the rational use of lipid-lowering drugs. *Drugs*, **2010**, *70*, 1363-79.

[3] Kostapanos, M.S.; Milionis, H.J.; Elisaf, M.S. Current role of statins in the treatment of essential hypertension. *Expert Opin. Pharmacother*., **2010**, *11*, 2635-50.

[4] Steinberg, D. Thematic review series: The pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy: part I. *J. Lipid Res*., **2004**, *45*, 1583-93.

[5] Gordon, T.; Casstelli, W.P.; Hjortland, M.C.; Kannel, W.B.; Dawber, T.R. High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. *American Journal of Medicine*, **1977**, *62*, 704-14.

[6] Ravnskov, U. Dietary fat is not the villain. *BMJ*, **2005**, *331*, 906-7.

[7] Olson, R.E. Discovery of the lipoproteins, their role in fat transport and their significance as risk factors. *J. Nutr.,* **1998**, *128*, 439S-443S.

[8] Vance D.E.; van den Bosch, H. Cholesterol in the year 2000. *Biochim. Biophys. Acta.*, **2000**, *1529*,1-8.

[9] Bloch, K. Sterol molecule: structure, biosynthesis, and function. *Steroids*, **1992**, *57*, 378-83.

[10] Tabas, I. Cholesterol in health and disease. *J. Clin. Invest.*, **2002**, *110*, 583-90.

[11] Fernandez, M.L.; West, K.L. Mechanisms by which dietary fatty acids module plasma lipids. *J. Nutr.* **2005**, *135*, 2075-78.

[12] Young, I.S. Lipids for psychiatrists - an overview. *J. Psychopharmacol*., **1995***, 19***,** 66-75.

[13] Mahley, R.W.; Innerarity, T.T.; Rall, S.C.; Weisgraber, K.H. Plasma lipoproteins: apolipoprotein structure and function. *J. Lipid Res.,* **1984***, 25*, 1277-94.

[14] Russell, D.W.; Setchell, K.D. Bile acid biosynthesis. *Biochemistry,* **1992**, *31*, 4737-49.

[15] Jessup W.; Kritharides, L.; Stocker, R. Lipid oxidation in atherogenesis: an overview. *Biochem. Soc. Trans.*, **2004**, *32*, 134-38.

[16] Brown M.S.; Goldstein, J.L. A receptor-mediated pathway for cholesterol homeostasis. *Science*, **1986**, *232*, 34-47.

[17] Fielding C.J.; Fielding, P.E. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.,* **1995**, *36*, 211-28.

[18] Barter P.J.; Kastelein, J.J. Targeting cholesteryl ester transfer protein for the prevention and management of cardiovascular disease. *J. Am. Coll. Cardiol*., **2006**, *47*, 492-99.

[19] Steinberg, D.; Fredrickson, D.S.; Avigan, J. Effects of Δ^4-cholestenone in animals and in man. *Proc. Soc. Exp. Biol.,* **1958**, *98*, 784-90.

[20] Steinberg, D.; Avigan, J.; Feigelson, E.B. Effects of Triparanol (Mer-29) on cholesterol biosynthesis and on blood sterol levels in man. *J. Clin. Invest.,* **1961**, *40*, 884-93.

[21] Tanzawa, K.; Kuroda, M.; Endo, A. Time-dependent, irreversible inhibition of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase by the antibiotic citrinin. *Biochem. Biophys. Acta.,* **1977**, *488*, 97-101.

[22] Endo, A. Compactin (ML-236B) and related compounds as potential cholesterol-lowering agents that inhibit HMG-CoA reductase. *J. Med. Chem.,* **1985**, *28*, 401-5.

[23] Robertson, J.G. Mechanistic basis of enzyme-targeted drugs. *Biochemistry*, **2005**, *44*, 5561-71.

[24] Shigematsu, H.; Hata, Y.; Yamamoto, M.; Oikawa, T.; Yamauchi, Y.; Nakaya, N.; Goto, Y. Treatment of hypercholesterolemia with an HMG-CoA reductase inhibitor (CS-500). Phase I study in normal subjects. *Geriat. Med. (Japan),* **1979**, *17*, 1564-73.

[25] Grundy, S.M. HMG-CoA reductase inhibitors for the treatment of hypercholesterolema. *N. Engl. J. Med.,* **1988**, *319*, 24 - 33.

[26] Endo, A. The origin of the statins. *International Congress Series*, **2004**, *1262*, 3-8.

[27] Corsini, A.; Maggi, F.M.; Catapano, A.L. Pharmacology of competitive inhibitors of HMG-CoA reductase. *Pharmacol. Res.,* **1995**, *31*, 9-27.

[28] Radulovic, L.L.; Cilla, D.D.; Posvar, E.L.; Sedman, A.J.; Whitfield, L.R. Effect of food on the bioavailability of atorvastatin, an HMG-CoA reductase inhibitor. *J. Clin. Pharmacol.,* **1995**, *35*, 990-94.

[29] Taillon, M.; Côté, C.; Furtado, M.; Garofolo, F. Potentially new isobaric metabolite of fluvastatin observed by LC-MS/MS during incurred sample analysis. *Bioanalysis*, **2011**, *3*, 1827-35.

[30] Davidson, M.H. Fluvastatin long-term extension trial (FLUENT): Summary of efficacy and safety. *Am. J. Med.,* **1994**, *96*, 41.

[31] Hamelin, B.A.; Turgeoun, J. Hydrophilicity/lipophilicity: relevance for the pharmacology and clinical effects of HMG-CoA reductase inhibitors. *Trends in Pharmacological Sciences,* **1998**, *19*, 36-37

[32] Ho, R.H.; Tirona, R.G.; Leake, B.F.; Glaeser, H.; Lee, W.; Lemke, C.J. Wang, Y.; Kim, R.B. Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology*, **2006**, *130*, 1793 - 1806.

[33] Bottorff, M.; Hansten, P. Long-term safety of hepatic hydroxymethyl glutaryl coenzyme A reductase inhibitors: the role of metabolism - monograph for physicians. *Arch. Intern. Med.*, **2000**, *160*, 2273-80.

[34] Tse, F.L.; Jaffe, J.M.; Troendle, A.; Pharmacokinetics of fluvastatin after single and multiple doses in normal volunteers*. J. Clin. Pharmacol.,* **1992**, *32*, 630-38.

[35] Schachter, M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fund. Clin. Pharm.*, **2004**, *19*, 117 - 25.

[36] Boberg, M.; Angerbauer, R.; Fey, P.; Kanhai, W.K.; Karl, W.; Kern, A.; Ploschke, J.; Radtke, M. Metabolism of cerivastatin by human liver microsomes in vitro. Characterization of primary metabolic pathways and of cytochrome P450 isozymes involved. *Drug Metab. Dispos.,* **1997**, *25*, 321-31.

[37] Sidaway, J.; Davidson, R.; McTaggart, F. Effect of statins on protein uptake and cholesterol biosynthesis in kidney proximal tubule cells. *Toxicol. Lett.,* **2003**, *144*, S96.

[38] Black, D.M. A general assessment of the safety of HMG CoA reducase inhibitors (statins). *Curr. Atherosclero. Rep*., **2002**, *4*, 34-41.

[39] Staffa, J.A.; Chang, J.; Green, J. Cervistatin and reports of fatal rhabdomyolysis. *N. Eng. J. Med.,* **2002**, *346*, 539-40.

[40] Ballantyre, C.M.; Corsini, A.; Davidson, M.H.; Holdaas, H.; Jacobson, T.A.; Leitersdorf, E.; März, W.; Reckless, J.P.; Stein, E.A. Risk for myopathy with statin therapy in high-risk patients. *Arch. Intern. Med.,* **2003**, *163*, 553-64.

[41] Jacobson, T.A. Statin safety: lessons from new drug applications for marketed statins. *Am. J. Cardiol.,* **2006**, *97*, 44C-51C.

[42] Istvan, E.S.; Palnitkar, M.; Buchanan, S.K.; Deisenhofer, J. Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. *EMBO J.,* **2000**, *19*, 849 - 60.

[43] Istvan, E.S.; Deisenhofer, J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*, **2001**, *292*, 1160 - 64.

[44] Straathof, A.J.J.; Panke, S.; Schmid, A. The production of fine chemicals by biotransformations. *Current Opinion in Biotechnology*, **2002**, *13*, 548-56.

[45] Vederas, J.C.; Moore, R.N.; Bigam, G.; Chan, K.J. Biosynthesis of the hypocholesterolemic agent mevinolin by *Aspergillus terreus*. Determination of the origin of carbon, hydrogen and oxygen by ^{13}C NMR and mass spectrometry. *J. Am. Chem. Soc.*, **1985**, *107*, 3694-701.

[46] Hoffman, W.F.; Alberts, A.W.; Anderson, P.S.; Chen, J.S.; Smith, R.L.; Willard, A.K. 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. 4. Side chain ester derivatives of mevinolin. *J. Med. Chem.,* **1986**, *29*, 849-52.

[47] Manzoni, M.; Rollini, M. Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. *Appl. Microbiol. Biotechnol.,* **2002**, *58*, 555-64.

[48] Witter, D.J.; Vederas, J.C. Putative Diels-Alder catalyzed cyclization during the biosynthesis of lovastatin. *J. Org. Chem.,* **1996**, *61*, 2613-23.

[49] Repič, O.; Prasad, K.; Lee, G.T. The story of lescol: from research to production. *Org. Process Res. Dev.*, **2001**, *5*, 519-27.

[50] Fuenfschilling, P.C.; Hoehn, P.; Mutz, J. An improved manufacturing process for fluvastatin. *Org. Process Res. Dev.*, **2007**, *11*, 13-18.

[51] Casar, Z. Straightforward and Efficient Synthesis of (4*R*,6*S*)-4-(*tert*-Butyldimethylsiloxy)-6-(hydroxymethyl) tetrahydropyran-2-one. *Synlett*, **2008**, 2036-40.

[52] Casar, Z.; Steinbücher, M.; Košmrlj, J. Lactone pathway to statins utilizing the Wittig reaction. The synthesis of rosuvastatin. *J. Org. Chem.,* **2010**, *75*, 6681-84.

[53] Richard, J.F; Christopher, D.; Mundorff, E.C.; Newman, L.M. Gavrilovic, V.; Ma, S.K. Chung, C.; Tam, S. Muley, S. Improving catalytic function by ProSAR driven enzyme evolution. *Nature Biotechnol*., **2007**, *25*, 338-44.

[54] Brower, P.L.; Butler, D.E.; Deering, C.F.; Le, T.V.; Millar, A.; Nanninga, T.N.; Palmer, C.W.; Roth, B.D. The convergent synthesis of (4*R*-*cis*)-1,1-dimethylethyl 6-cyanomethyl-2,2-dimethyl-1,3-dioxane-4-acetate, a key intermediate for the preparation of CI-981, a highly potent, tissue selective inhibitor of HMG-CoA reductase. *Tetrahedron Lett*., **1995**, *33*, 2279-82.

[55] Takemoto, M.; Liao, J.K. Pleiotropic effects of 3-hydroxy-3-methylglutarylcoenzyme a reductase inhibitors. *Arterioscler. Thromb. Vasc. Biol.,* **2001**, *21*, 1712-19.

[56] Aviram M.; Rosenblat, M.; Bisgaier, C.L.; Newton, R.S. Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. *Atherosclerosis,* **1998**, *138*, 271-80.

[57] Davignon, J.; Jacob, R.F.; Mason, R.P. The antioxidant effect of statins*. Coronary Artery Disease*, **2004**, *15*, 251-58.

[58] Hobson, L.A.; Akiti, O.; Deshmukh, S.S.; Harper, S.; Katipally, K.; Lai, C.J.; Livingston, R.C.; Lo, E.; Miller, M.M.; Ramakrishnan, S.; Shen, L.; Spink, J.; Tummala, S.; Wei, C.; Yamamoto, K.; Young, J.; Parsons, R.L. Development of a scalable process for the synthesis of a next-generation statin. *Org. Process Res. Dev.*, **2010**, *14*, 441-58.

[59] Zhang, Q.Y.; Wan, J.; Xu, X.; Yang, G.F.; Ren, Y.L.; Liu, J.J.; Wang, H.; Guo, Y. Structure-based rational quest for potential novel inhibitors of human HMG-CoA reductase by combining CoMFA 3D QSAR modelling and virtual screening. *Journal of Combinatorial Chemistry*, **2006**, *9*, 131-38.

[60] Barter, P. CETP and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.,* **2000**, *20*, 2029-31.

[61] Tall, A.R.; Yvan-Charvet, L.; Wang, N. The failure of torecetrapib. Was it the molecule or the mechanism? *Arterioscler. Thromb. Vasc. Biol*., **2007**, *27*, 257-60.

[62] Cannon, C.P.; Shah, S.; Dansky, H.M.; Davidson, M.D.; Brinton, E.A.; Gotto, A.M.; Stepanavage, M.; Liu, S.X.; Gibbons, P.; Ashraf, T.B.; Zafarino, J.; Mitchel, Y. Barter, P. Safety of Anacetrapib in Patients with or at High Risk for Coronary Heart Disease. *N. Engl. J. Med.,* **2010**, *363*, 2406-15.

[63] Rosenson, R.S. Rosuvastatin: a new inhibitor of HMG-CoA reductase for the treatment of dyslipidemia. *Expert Rev. Cardiovasc. Ther.,* **2003**, *1*, 495-505.

[64] Olsson, A.G.; Pears, J.; McKellar, J.; Mizan, J.; Raza, A. Effect of rosuvastatin on low-density lipoprotein cholesterol in patients with hypercholesterolemia. *Am. J. Cardiol.,* **2001**, *88*, 504-8.

[65] Saito Y.; Yamada N.; Teramoto T.A. randomized double-blind trial comparing the efficacy and safety of pitavastatin versus pravastatin in patients with primary hypercholesteremia. *Atherosclerosis,* **2002**, *162,* 373-79.

[66] Black D.M. A general assessment of the safety of HMG CoA reductase inhibitors (statins). *Curr. Atheroscler. Rep.,* **2002**, *4*, 34-41.

Received: November 11, 2011 Revised: March 25, 2012 Accepted: March 28, 2012