

## TEACHERS' TOPICS

### Antihyperlipidemic Statins: A Self-Contained, Clinically Relevant Medicinal Chemistry Lesson

Victoria F. Roche, PhD

School of Pharmacy and Health Professions, Creighton University Medical Center

Submitted December 14, 2004; accepted January 23, 2005; published September 13, 2005.

Medicinal chemistry instruction at Creighton University is designed to provide an in-depth, scientifically grounded and clinically relevant learning experience for pharmacy students. Each topic covered in the 2-semester required course sequence is selected based on the general utility of the compounds in question and/or the therapeutic importance of the drugs in treating life-threatening diseases. All lessons provided to campus- and Internet-based students by the author are in the form of a descriptive and conversational narrative, and course requirements are in place to assure that students read the lesson prior to the class period in which it is discussed. Learning tools and aids are provided to help students readily discern the most critical aspects of each lesson, to practice required critical thinking and analysis skills, and to self-assess competency in meeting specific learning objectives. This manuscript illustrates this approach by sharing a lesson on the chemistry and clinically relevant structure-activity relationships of antihyperlipidemic statins, and the tools utilized to optimize learning of the lesson's important chemical and therapeutic messages.

**Keywords:** medicinal chemistry, dyslipidemia, hyperlipidemia, statins

#### INTRODUCTION

At Creighton University, medicinal chemistry content is offered to second-year pharmacy students through 2 sequential courses entitled *The Chemical Basis of Drug Action I* and *II*. The fall semester's course is a 3-credit hour experience, while that offered in the spring is a 2-credit hour course. One philosophy, list of learning objectives, and set of guiding principles underpins and shapes both courses. A practice-oriented approach, which emphasizes the relevance of chemistry to the contemporary practice of pharmacy, was purposefully implemented to give students the skills necessary to predict biological properties and therapeutic activities of current and future drug molecules.<sup>1-4</sup> The course sequence builds upon previously acquired knowledge of biochemistry, pharmaceuticals, and basic organic chemistry and pharmaceutical sciences principles, complements concepts being addressed concomitantly in pharmacology, and prepares students for therapeutics course work in the third-professional year.

The *Chemical Basis* course sequence builds from an introduction (or re-introduction) of acid-base chemistry

taught from a structural perspective. Functional group chemistry, drug receptor structure and signal transduction pathways, and drug metabolism are addressed before moving into the discussion of drug classes. The drug structures covered in the fall semester are, in general, smaller and less mechanistically complex than those addressed in the spring. "Starting small" allows students to gain confidence in their ability to dissect drug structure and translate it into anticipated pharmacological action, and not become overwhelmed by the larger, more chemically intricate structures found in the antibiotic antineoplastics, opioids, or steroid classes covered in the second semester. Students learn to recognize key functional groups and, simultaneously, evaluate the entire drug structure with respect to anticipated receptor affinity, binding selectivity, distribution, metabolism, and excretion patterns. Finally, they learn to use their understanding of drug action, gained through a thorough mechanistic analysis of drug structure and chemistry, to make and defend therapeutic choices for individual patients.

#### CHEMICAL BASIS LESSONS & LEARNING TOOLS

Each topic covered by this author in the *Chemical Basis* courses is delivered to students in the form of 1 or more conversational lesson handouts. The lesson handouts were constructed to be totally self-explanatory when the Internet-based first-professional degree Doctor of

---

**Corresponding Author:** Victoria F. Roche, PhD. Address: Senior Associate Dean, Professor of Pharmaceutical Sciences, School of Pharmacy and Health Professions, Creighton University Medical Center, 2500 California Plaza, Omaha, NE 68178. Tel: 402-280-3191. Fax: 402-280-5692. E-mail: roche@creighton.edu

Pharmacy program was introduced. Although the class lecture and learning activities are audiotaped for use by the Internet-based students, they have no visual connection with the classroom. Chemistry is a highly visual science, and it was felt that students learning from a distance would be disadvantaged unless the lesson materials were descriptive and accommodating of the visual learning tendencies of many of our pharmacy students. The relative success of the campus- and Internet-based students in achieving performance and learning parity in the *Chemical Basis* courses has been addressed in a separate article.<sup>5</sup>

All lesson handouts are divided into discrete sections, including:

- A brief introduction to the topic,
- Pertinent review (eg, biochemical pathways impacted by the drugs under study and the pharmacological mechanism of action),
- The chemical nature of the binding site,
- Drug-receptor binding graphics,
- A receptor-based discussion of the pharmacophore,
- Structure activity relationships (SAR),
- Pathways of metabolic activation/inactivation, and
- Therapeutic agents and clinical correlates

“Brain Teaser” questions are utilized sparingly throughout the lesson to stimulate thinking and keep students engaged in the content.

Specific learning objectives are written for each *Chemical Basis* lesson and provided to the students via the course website. Owing to the conversational nature of the lessons, the handouts can be quite long and many are highly detailed. To assist students in homing in on the key elements of each lesson, a summary of the most important concepts or “take home” messages, entitled, “Med. Chem. To Go,” is also linked to the course website.

Students are required to read the lesson handout prior to the class period in which the material will be formally presented and discussed. This requirement is actualized through the offering of weekly on-line open book “pre-class” reading assessment quizzes. Quiz questions address key chemical concepts and knowledge of SAR as described in the lesson handout and, on occasion, the application of SAR to a clinical situation. Since the quizzes are designed to prompt students to read the lesson and come to class prepared for discussion (rather than to assess mastery of content), students may refer to the lesson handout and/or their text while they are taking their quiz. They are encouraged to work in groups so that potential answers to quiz questions can be discussed

and/or debated and learning can be reinforced. While group work is permitted in preparing for the quiz, each student must complete and submit her/his own quiz electronically to receive credit. Eleven or twelve quizzes are given each semester and, to reward students for proactive preparation, the quiz average counts for 10% of each student's final course grade. There is no reason why a student should not earn perfect scores on each quiz, so the reward for preparedness can be as much as a letter grade.

Once students have reviewed the lesson objectives and “Med. Chem. To Go” documents, read the lesson handout and the recommended pages of the optional text, and participated in (or listened to) the in-class discussion, they are ready to try their hand at some application exercises. *Chemical Basis* examinations require students to demonstrate a high level of understanding, analysis, application, and content mastery, so optional structure challenge exercises, study questions, problem worksheets, case studies, and practice examinations that assist students in skill development are a “must.” Faculty members are willing to discuss these exercises with students individually or go through them in the weekly voluntary recitation period. Students are encouraged to work the problems and bring their papers in for a personal consultation on performance strengths and weaknesses. Internet-based students can receive a telephone consult on these optional learning activities after first faxing their work, or submitting it electronically, to the faculty member. The exercises are available for students on the course website at least 1 week (and usually several weeks) prior to when the lesson is covered in class. However, to encourage students to work in an intellectually independent fashion on the exercises, the answer keys are posted only a week or so before the pertinent examination. Students requesting an earlier posting of the key are reminded that there is a world of difference between recognizing a right answer when it is written out for you and being able to generate a right answer de novo, and that the only way to master analytical and application skills is to practice.

Some of the lessons covered in the *Chemical Basis* courses have computerized medicinal chemistry case studies associated with them.<sup>6-7</sup> The computerized cases are complex decision trees that present a patient-specific scenario requiring a therapeutic decision along with 4 to 5 potential drug candidate structures. Questions addressing desired or anticipated therapeutic outcomes are posed and students select answers that give chemically based explanations for clinical responses. Drug names are not revealed until the end of the appropriate algorithm arm so that chemical reasoning motivates student

response to case questions. Wrong responses generate additional questions that guide students in correcting their mistakes, while correct answers are positively reinforced. Humor and graphics are utilized liberally to engage learners.

Currently, computerized case studies are available for the  $\alpha$ - and  $\beta$ -adrenergic agonists, opioid analgetics, H1 antagonists and antihyperlipidemics, and they have been well received by Creighton pharmacy students. When available, these cases are used in class as a fun, summative active-learning exercise. Those cases not previously published commercially are made available on the course website for all to access at their leisure. A compact disc of the 2 computerized cases not commercially available ("Botswana: An Opioid Adventure" and "The Freak of Nature: A Psychedelic Antihyperlipidemic Experience") can be obtained at no charge from the author.

To illustrate the above, the handout made available to students for the "Antihyperlipidemic Statins: Chemistry and SAR" lesson is provided. A copy of the companion lesson "Antihyperlipidemic Statins: Therapeutic Agents and Clinical Considerations" can be obtained from the author. While the handout is not referenced, students are made aware that lesson material comes information gleaned from the antihyperlipoproteinemics chapter in the recommended textbook<sup>8</sup> and from the scientific and clinical literature.<sup>9-29</sup> The learning objectives, study questions, and a sample optional learning exercise for this lesson are provided in Appendix 1,2, and 3, respectively. The "Med. Chem. To Go" document, pre-class reading assessment quiz, and additional antihyperlipidemic statins optional-learning exercises (including case studies) can be obtained from the author.

## **ANTIHYPERLIPIDEMIC STATINS LESSON**

### **Introduction**

As a regulator of homeostasis, a precursor to the corticosteroids and sex hormones, and a critical factor in the maintenance of cell wall integrity, cholesterol is essential to life. However, high levels of this lipophilic substance lead to atherosclerosis, a predisposing factor to the development of coronary artery disease (CAD). Atherosclerosis involves an accumulation of cholesterol esters and other blood lipids and lipoproteins in macrophage cells found in the intima of arteries. Lipid-engorged macrophage cells become foam cells, and foam cell infiltration progresses to fatty streaks in the arterial wall. Plaque formation, thrombosis, and vessel occlusion can follow, leading to CAD. CAD involves one or more specific cardiovascular pathologies, including myocardial infarction, ischemia, and angina. Between 13 and 14 million people in the

United States are believed to suffer from this complex and life-threatening condition, and over 25 million people worldwide are expected to die from cardiovascular-related pathologies by the year 2020.

In addition to free cholesterol and its esters, triglycerides (long-chain fatty acid esters of the polyalcohol glycerol) and lipoproteins (macromolecular substances that solubilize blood lipids) are found in the bloodstream. High levels of triglycerides and the lipid-rich lipoproteins that promote the formation of atherosclerotic plaques (low density lipoproteins [LDL] and very low density lipoproteins [VLDL]) are also a significant health risk in developed nations where lifestyles are sedentary, stress is "sky high," and fat-laden meals are too often the norm. Patients with elevated levels of triglycerides and "bad cholesterol" are at risk for myocardial infarction and/or cerebral vascular accident (stroke).

Serum cholesterol comes from both exogenous (dietary) and endogenous (biosynthetic) sources, so following a low-fat diet and exercising regularly can keep serum lipid levels in check for many. However, an individual's specific biochemical and metabolic profile can often work against even the healthiest lifestyle. For these "biochemically challenged" patients, lipid-lowering agents such as the statins have literally provided a new lease on life.

### **Cholesterol Biosynthesis and Metabolism**

[The cholesterol biosynthetic pathway is presented at this point, and the scheme is briefly summarized].

The big "take home" message of this synthetic pathway for us is found in the stereospecific conversion of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) to (R)-mevalonic acid, which is catalyzed by the enzyme HMG CoA reductase (commonly known as HMGR). As the rate-limiting enzyme, it is the therapeutic linchpin in cholesterol biosynthesis, and the site of action of the most popular of the lipid-lowering agents, the statins (Figure 1).

To understand how the statins work to block HMG CoA reductase, we need to learn more about how this enzyme binds its natural substrate, HMG CoA. So, let's do it!

### **HMG CoA Reductase Chemistry**

HMG CoA reductase (HMGR) catalyzes the rate-limiting step in cholesterol biosynthesis. The reduction of 3-hydroxy-3-methylglutaric acid (HMG) to mevalonic acid involves the transfer of 4 electrons (via 2 molecules of NADPH cofactor) to a substrate that has been activated for reaction with the sulfhydryl (SH) containing coenzyme A (designated as CoASH).

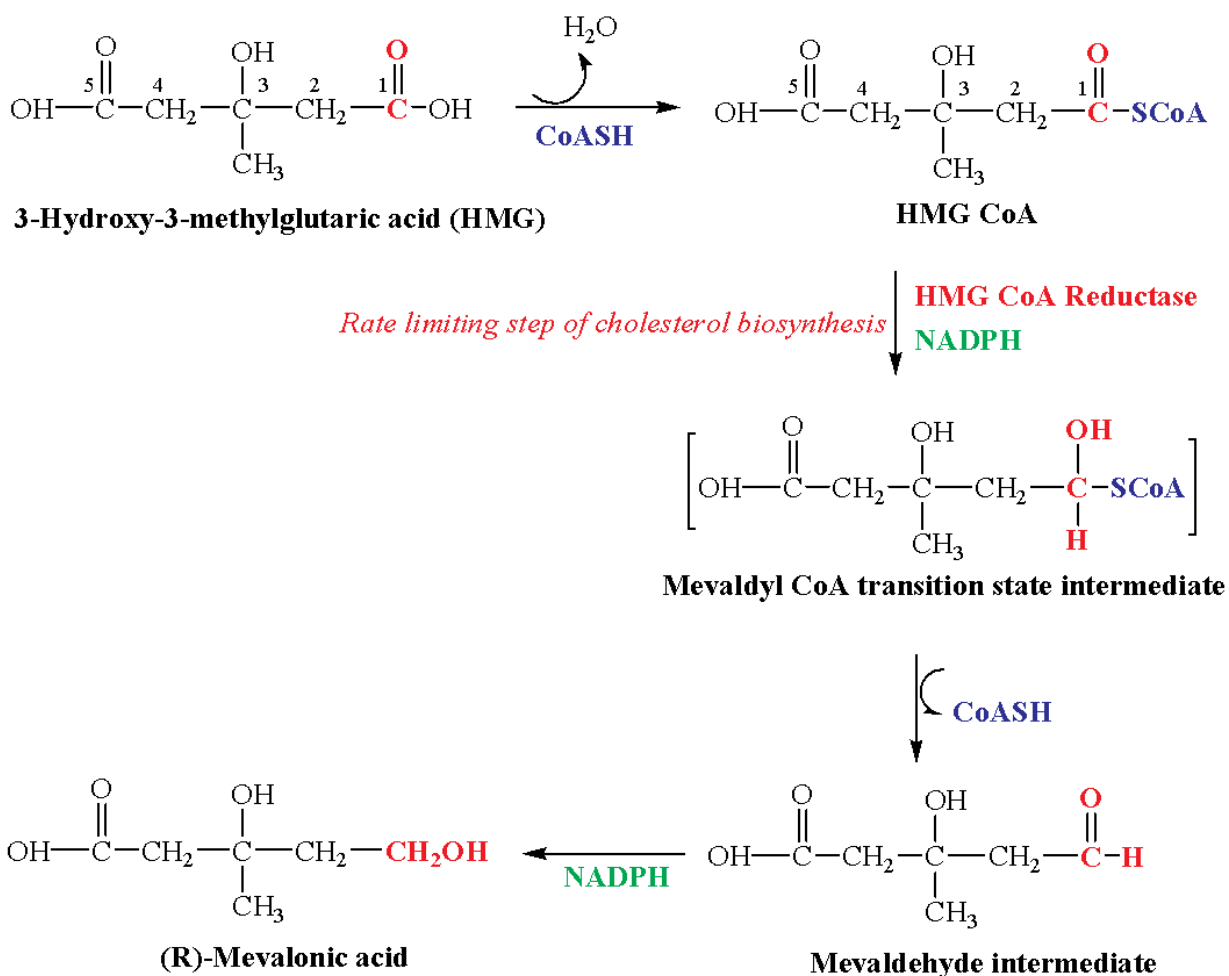


Figure 1. Mevalonic acid biosynthesis.

The binding site for the HMG CoA substrate is of critical importance to this lesson. Some of the key amino acid residues of HMGR that bind to the HMG CoA substrate have been identified. These include:

- Lys735 (in cationic conjugate acid form), which anchors the substrate to the enzyme through an ion-ion bond with the C5 anionic carboxylate group of HMG CoA.
- Lys692, also in cationic form, which stabilizes the carbonyl oxygen of this group through an ion-dipole bond.
- The C3-OH group of the substrate, which is stabilized by two residues, Ser684 and Asp690. Serine acts as an H-donor in a H-bond with the oxygen of the OH group, and anionic Asp forms an ion-dipole bond with the alcoholic hydrogen.
- Lys691 (in cationic conjugate acid form), which engages in an ion-dipole bond with the carbonyl oxygen of C1. Remember that this is the carbonyl group that will be reduced to the primary alcohol through the action of the HMGR

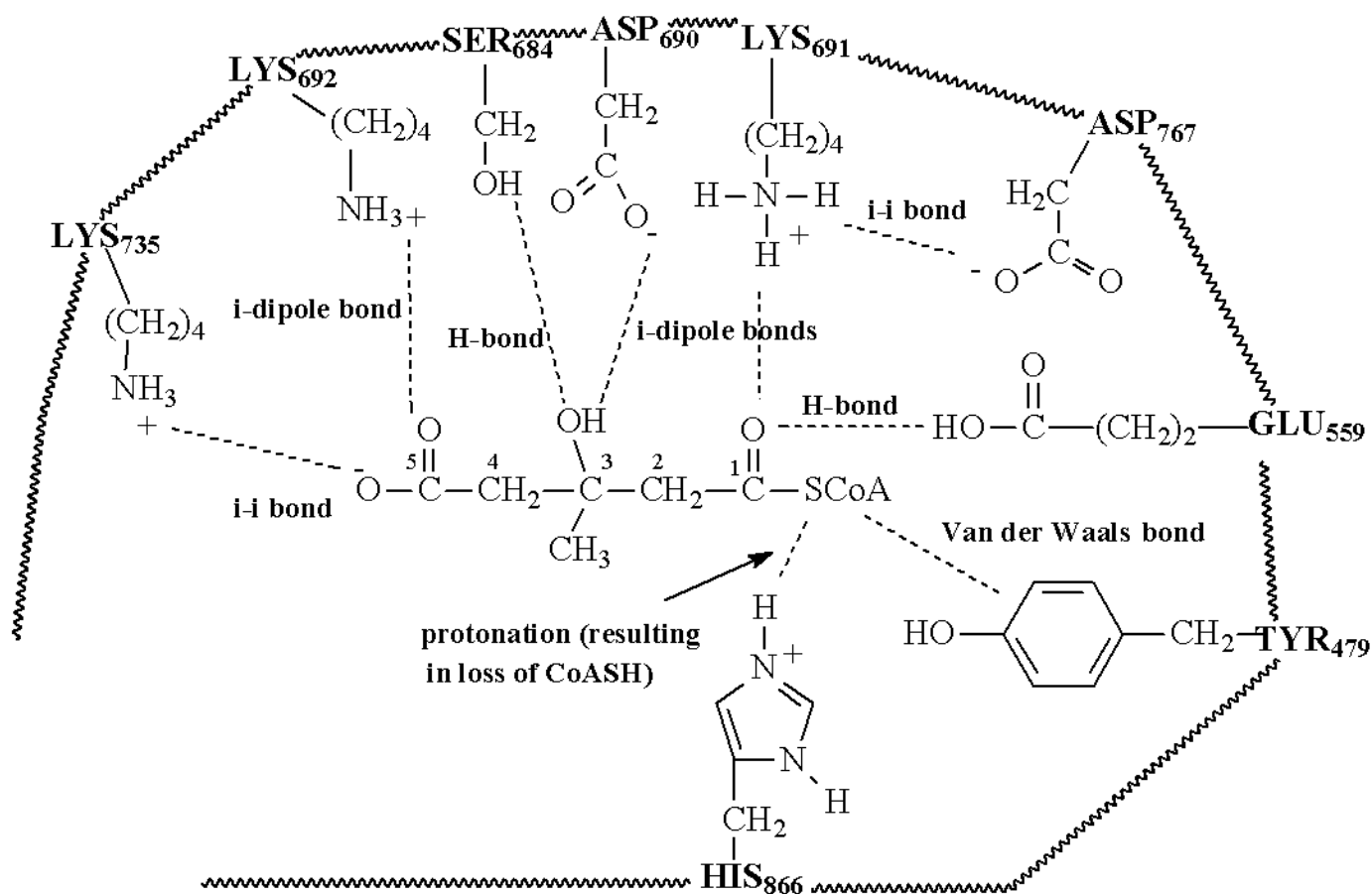
enzyme. Lys691 is found in a region (or domain) of the receptor referred to as the cis loop.

- An anionic Asp767, which promotes this important ion-dipole interaction by forming its own ion-ion bond with the Lys residue. This interaction stabilizes the cation for interaction with the HMG CoA substrate.
- Glu559 (in unionized acid form), which also hydrogen bonds to the same C1 carbonyl oxygen. By forming these 2 important bonds, this carbonyl group is held tightly to the enzyme, and is properly oriented for the reduction to come.

In addition to these interactions, Tyr479 engages in a Van der Waals bond with the adenine base of the CoA portion of the substrate, forming a kind of “hydrophobic shield” that closes the binding pocket down for effective reduction by the NADPH cofactor.

During the first NADPH reduction, the doubly bonded nitrogen of His866 (in cationic conjugate acid form) acts as a hydrogen donor to the sulfur atom of the thioester (SCoA), which liberates CoASH from the sub-





## HMG CoA Reductase binding interactions with the endogenous HMG CoA substrate

Figure 2. HMGR binding interactions with the endogenous HMG CoA substrate.

strate. This, in turn, produces the mevaldehyde intermediate. Check out the diagram illustrating the binding of the endogenous substrate, HMG CoA with the HMGR enzyme (Figure 2).

### HMGR Inhibitor (Statin) SAR

The statins are competitive antagonists of HMG CoA. They compete directly with the endogenous substrate for the active site cavity of HMGR. The commercially available statins are analogs of natural HMGR inhibiting substances isolated from *Penicillium citrinum* and other natural products. Three statin products (lovastatin, simvastatin, and pravastatin) are either isolated from, or generated from, fungi (*Aspergillus terreus* and *Monascus ruber*).

Statins exert their therapeutic effect primarily by inhibiting cholesterol biosynthesis and by stimulating the receptor-mediated uptake of LDL, which results in a lowering of serum LDL levels. The different statins have a variable impact on HDL cholesterol elevation, but all are able to lower triglyceride levels to some extent.

### The Statin Pharmacophore

All statins consist of 2 specific structural components, a dihydroxyheptanoic acid unit and a ring system with lipophilic substituents. The dihydroxyheptanoic acid component is essential to HMGR-inhibiting activity. Since they bind to the same active site, the structure of the statin HMGR inhibitors resembles the endogenous substrate, HMG CoA.

All statins contain a modified hydroxyglutaric acid component that mimics the 3-hydroxyglutaryl unit of both the substrate (HMG CoA) and the mevaldyl CoA transition state intermediate (Figure 3). See for yourself!

Statins that are active intact contain a free acidic functional group, which mimics the free COOH of HMG CoA. These compounds exist as anions at pH 7.4, and this is critical to their ability to compete for the HMGR active site by anchoring via an electrostatic bond to the cationic Lys735 of the enzyme.

Compounds that have their carboxylic acid group "tied up" in a lactone (cyclic ester) are prodrugs. The essential anionic carboxylate group must be liberated by

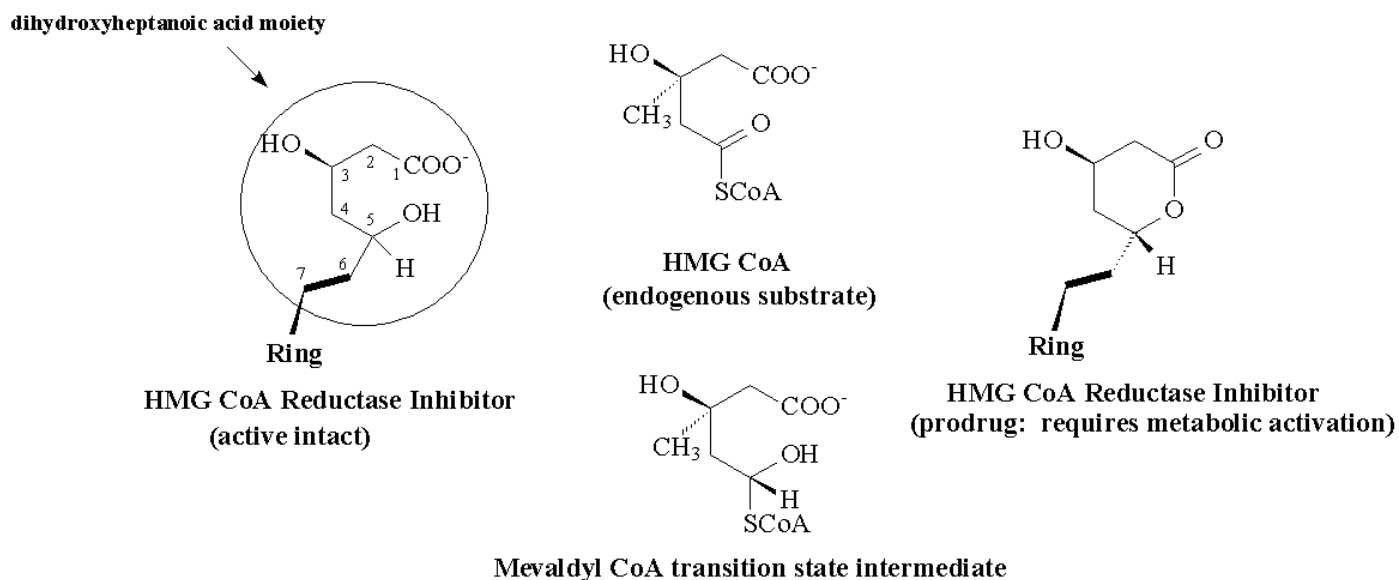


Figure 3. Structural relationship between HMG CoA and the statin pharmacophore.

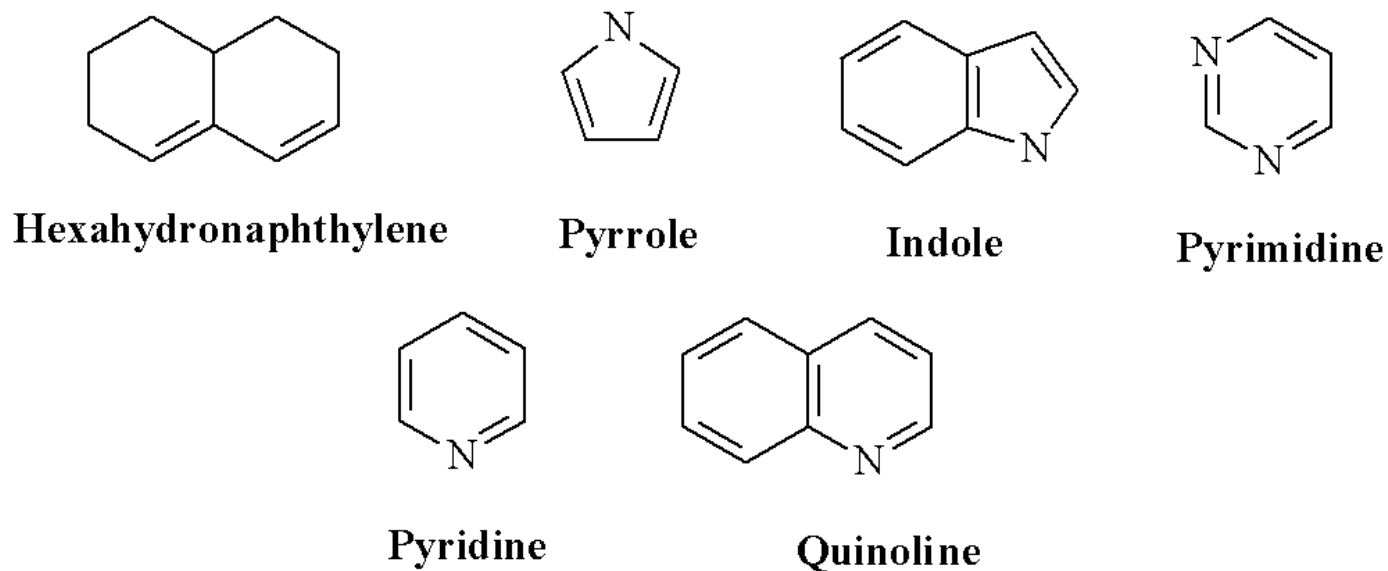


Figure 4. Ring systems employed in clinically useful HMGR inhibitors.

hydrolysis before activity is realized. Of course, the enzymes that catalyze the activating hydrolysis reaction are esterases.

The HMGR enzyme is stereoselective. Note that C3 and C5 of the dihydroxyheptanoic acid segment (the carbons that bear the OH groups) are chiral. The receptor preferentially binds the 3R,5R isomer of the stains. All marketed statins have this required stereochemistry.

The ring component of the HMG CoA reductase inhibitors binds to the HMGR enzyme in the same general area where the coenzyme A component of the endogenous HMG CoA substrate binds. The binding interactions in this area hold the inhibitor very tightly to the enzyme. This, in turn, decreases the chance that the

endogenous HMG CoA substrate will displace the inhibitor from the binding site. Ring systems (Figure 4) utilized in clinically useful HMGR inhibitors include:

- Partially reduced naphthylene (lovastatin, simvastatin, pravastatin)
- Pyrrole (atorvastatin)
- Indole (fluvastatin)
- Pyrimidine (rosuvastatin)
- Pyridine (glenvastatin, investigational)
- Quinoline (pitavastatin, investigational)

Regardless of the type of ring used, there will be at least 2 lipophilic substituents affixed to it. Curious about what the chemistry of these lipophilic groups will be? Read on!

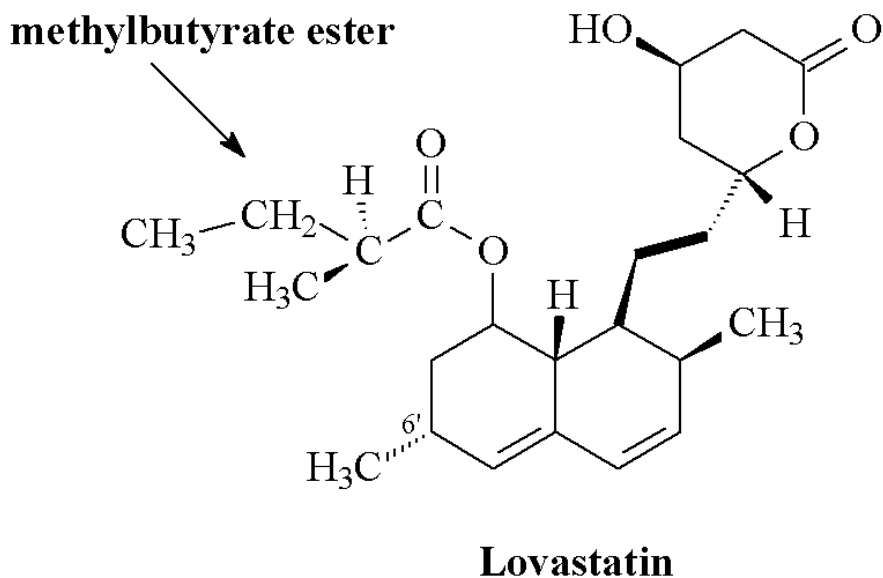


Figure 5. Methylbutyrate ester of lovastatin.

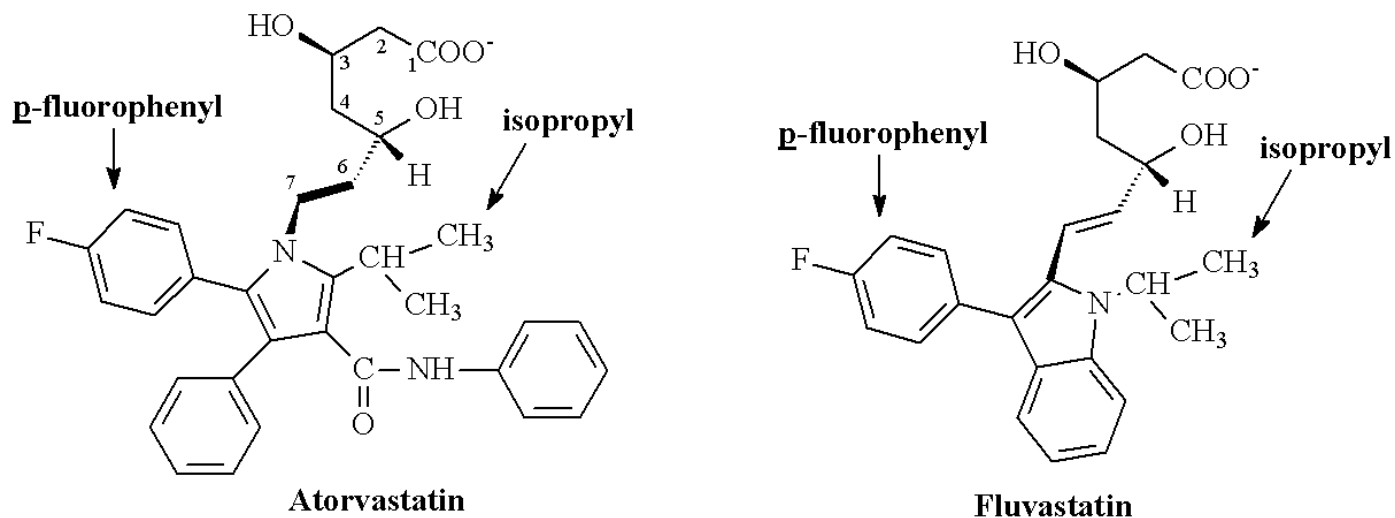


Figure 6. Functional groups common to atorvastatin and fluvastatin.

Statins that contain a partially reduced naphthylene ring system all have 1 or 2 CH<sub>3</sub> groups, and 1 methylbutyrate ester substituent on the ring (eg, lovastatin, Figure 5). The  $\alpha$ -CH<sup>3</sup> group at position 6' on the reduced naphthylene ring of these statins enhances HMGR inhibiting activity twofold over the unsubstituted derivative (which only has H at this position).

**Brain Teaser:** *Is lovastatin a prodrug, or is it active as administered? What is the structural basis for your answer?*

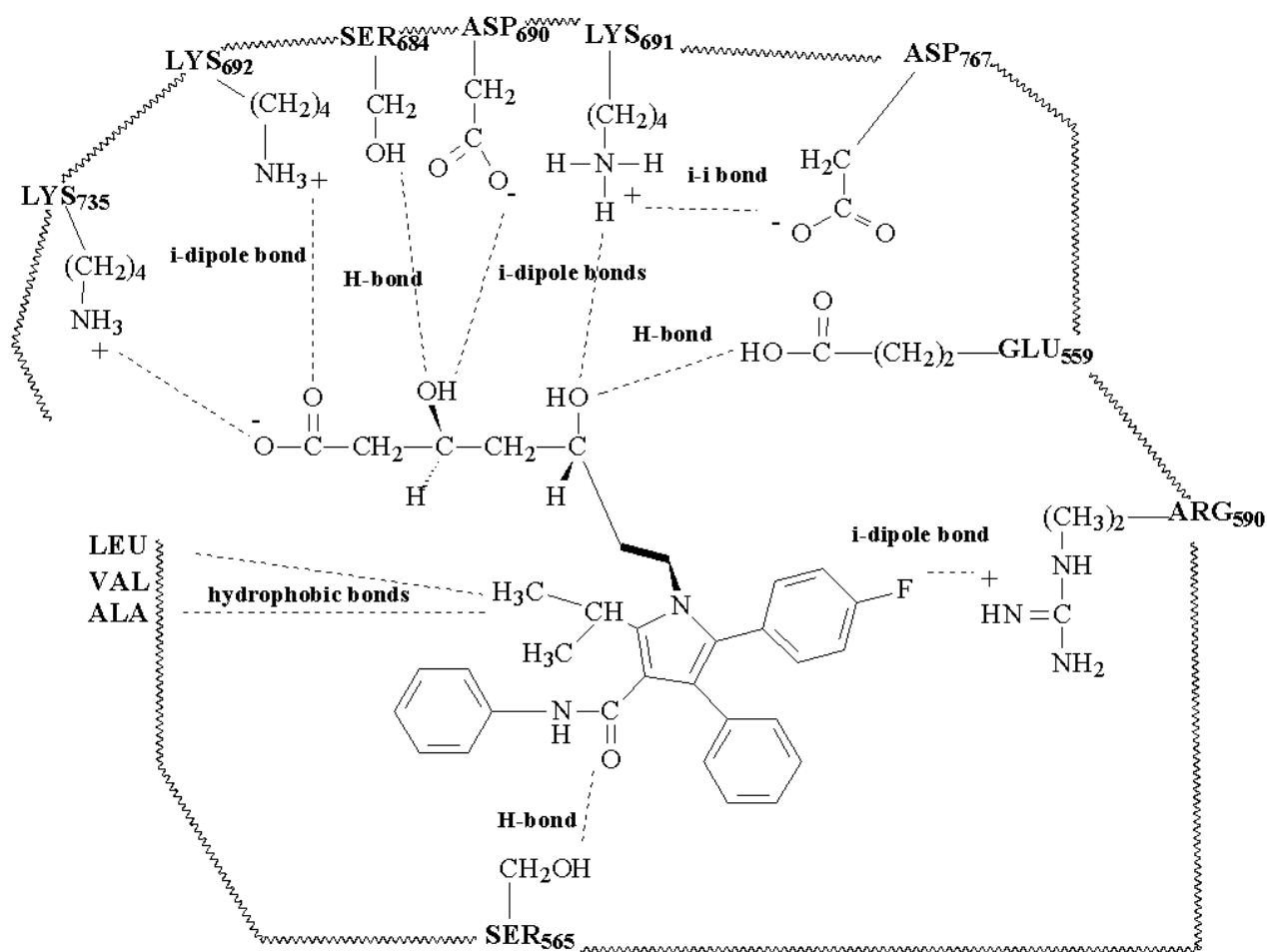
Statins that have nitrogen-containing ring systems all have isopropyl and p-fluorophenyl substituents attached to their rings. (eg, atorvastatin and fluvastatin) The p-fluorophenyl group takes up about the same

amount of space as the methylbutyrate ester group, and they both add lipophilicity to the structure. Check out the structures of atorvastatin and fluvastatin (Figure 6).

#### Statin Binding to HMGR

The binding of the dihydroxyheptanoic acid portion of the statins to the HMGR precisely mimics that of the endogenous substrate HMG CoA. Electrostatic, ion-dipole, and hydrogen bonds are all involved. Specifically:

- The anionic C1 carboxylate oxygen atom anchors to Lys735 via an ion-ion bond.
- Lys692 binds with the second (carbonyl) oxygen of this carboxylate group in an ion-dipole bond



### HMG CoA Reductase binding interactions with atorvastatin

Figure 7. HMGR binding interactions with atorvastatin.

(where Lys is cationic and the carbonyl oxygen is  $\Delta^-$ )

- Anionic Asp690 interacts in an ion-dipole bond with hydrogen of the C3-OH group.
- Ser684 acts as a H-donor in a H-bond with the oxygen atom of the same C3-OH
- Lys691, Glu559 and Asp767 interact in a coordinated network of ionic and hydrogen binding with the C5-OH group, just like they did with the carbonyl oxygen of the HMG CoA substrate.

Here is what is different. The statins take advantage of the flexibility of the HMGR receptor, enticing it to accommodate their large, lipophilic ring systems and substituents. These bulkier groups distort the active site cavity to form a shallow, hydrophobic pocket that binds tightly with these groups. (Think of the binding area for these large lipophilic rings like a big bean-bag chair, which readily changes shape to match whatever is trying to occupy it). Some specific statin-HMGR interactions are described below.

- The  $\Delta^-$  F atom of the fluorophenyl group interacts

with a cationic Arg590 in an ion-dipole bond.

- Hydrophobic interactions between the carbon rich methylbutyrate, isopropyl and methyl substituents on the statin rings occur with Leu562, Val683, Leu853, Ala856, and Leu857 residues on the enzyme.
- The carbonyl oxygen of the unique amide sidechain of atorvastatin and the sulfonamide sidechain of rosuvastatin is involved in a H-bond with Ser565

The additional binding interactions provided by the bulky rings and lipophilic substituents gives the statin inhibitors an affinity for the HMGR enzyme that is between  $10^3$  and  $10^4$  times higher than that of the endogenous substrate, HMG CoA.

This makes them very effective inhibitors, since HMG CoA has a very difficult time competing for access to its usual binding site. Of course, if HMG CoA can not be reduced to mevalonic acid, the de novo synthesis of cholesterol stops (Figure 7).



**Brain Teaser:** *Dr. Philip Portoghese, a renowned medicinal chemist from the University of Minnesota, developed a concept called “Message-Address” which conceptually breaks a molecule up into 2 components...one which “finds” the active site (the address) and the other which actually delivers the drug’s chemical message. Which segment of the statin’s structure would you classify as the address component, and which would be the message?*

### **Physicochemical Properties of Statins**

The relative lipophilicity of the statins plays a major role in their pharmacological activity profile and therapeutic utility. Four of the 6 statins are classified as lipophilic (lovastatin, simvastatin, atorvastatin, and fluvastatin) and 2 are classified as more hydrophilic in nature (pravastatin and rosuvastatin).

In general, the more lipophilic statins:

- Have a relatively lower hepatoselectivity than the hydrophilic statins. In other words they do not selectively distribute to the liver to as great an extent.

All statins are given orally, and the lipophilic statins will be readily extracted by the liver on first pass. This is good, as the liver is the site of cholesterol biosynthesis and, therefore, the target organ for the statins. Because of their high penetration into hepatocytes, some hepatoselectivity is observed from all of the lipophilic statins. However, since they came into the hepatocytes via passive diffusion, they can penetrate right back out of them, too. Once in the general circulation, their lipophilicity makes them very capable of passively penetrating into the cells of extrahepatic tissues, leading to side effects, some of which are undesired while others are desired. We will expound more on that later.

In contrast, it has been proposed that the more hydrophilic statins are extracted into the liver by a “one way” carrier-mediated active transport system. They tend to stay in the liver after they are extracted on first pass because they are too hydrophilic to distribute out via passive diffusion. For the same reason, these drugs do not penetrate well into the cells of other tissues. Therefore, the hydrophilic statins show a higher hepatoselectivity than the lipophilic statins. Pravastatin and rosuvastatin are the 2 most hydrophilic of the statins, and they have been shown to be the most hepatoselective, ie, they:

- Bind more extensively to serum proteins than the hydrophilic statins.
- Have a higher incidence of adverse effects. As mentioned, the more lipophilic statins are more likely to “escape” the liver after being extracted

out of the blood on first pass, and equally likely to penetrate the membranes of extrahepatic cells, leading to unwanted and potentially harmful side effects (eg, myalgia, rhabdomyolysis). Some authors claim that a firm correlation between lipophilicity and adverse effects has not been established, and caution us to take protein binding and the rate of metabolic degradation into account when evaluating the potential for adverse effects from the various statins.

- Are better able to sensitize smooth muscle cells to apoptosis (programmed cell death). This leads to a decreased stability of atherosclerotic plaque, which may be good news in mild disease, as plaque thinning and decreased arterial occlusion can result. However, it can be harmful in severe disease, due to an increased risk of dislodging a potentially fatal thrombus when the plaque thins.
- Can better penetrate into the CNS, and can reduce the incidence of Alzheimer’s dementia by decreasing central levels of certain  $\beta$ -amyloid peptides associated with this disease. This is one “side effect” of statin use that may be welcomed. The prodrugs lovastatin and simvastatin are the most lipophilic of all the statins since they lack the anionic carboxylate group in parent drug form. These 2 lipophilic statins have shown particular therapeutic promise in protecting against the development of Alzheimer’s Disease.

### **Statin Metabolism**

The prodrug statins lovastatin and simvastatin, which have their 3,5-dihydroxyheptanoic acid segment cyclized into a lactone ring, must be activated by hydrolysis to the active, anionic carboxylate form. Only when hydrolyzed can these agents anchor to the cationic Lys735 of the HMGR enzyme

CYP3A4 and CYP2C9 are both involved in the oxidative metabolism of the various statins, but there is little consistency in the chemical nature or pharmacological activity of the metabolites. The metabolic fate and the impact of that metabolism on potency and duration of action must be evaluated separately for each statin drug. Briefly:

- CYP3A4 is the predominant CYP isoform involved in the metabolism of atorvastatin, lovastatin, and simvastatin. [It was also the isoform that metabolized cerivastatin (by demethylation and hydroxylation to active metabolites). Cerivastatin was pulled from the market in 2001 for excessive toxicity].

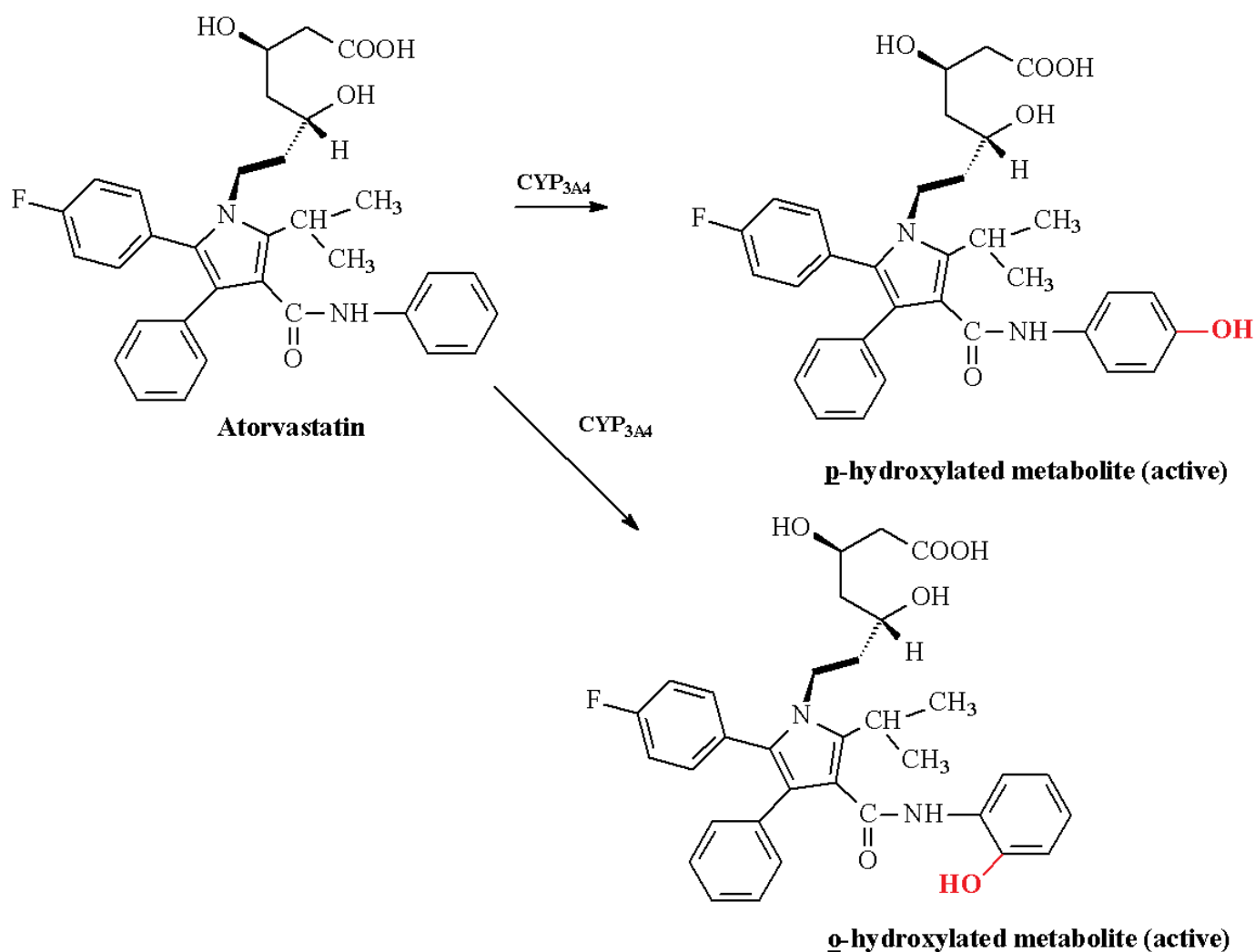


Figure 8. Atorvastatin metabolism.

The CYP3A4-mediated reactions on these statins include:

- Atorvastatin (Figure 8): aromatic hydroxylation at the *p* and *o* positions of the phenyl ring connected to the carboxamide group. These metabolites are both active.
- Lovastatin and Simvastatin (Figure 9): hydroxylation of the reduced naphthylene ring at the 6'-position, and at the 3-position of the methylbutyrate sidechain. These metabolites are inactive.

There is a significant drug-food interaction between the CYP3A4 statins and grapefruit juice, which inhibits this enzyme in the intestinal mucosa and liver. Increased toxicity has been noted when these drugs are consumed with grapefruit juice.

- CYP2C9 is the predominant isoform involved in the metabolism of fluvastatin.
- CYP2C9 hydroxylates the indole ring of fluvastatin at C5 and C6 to produce active metabolites. Fluvastatin also undergoes CYP2C9-mediated dealky-

lation of the indole nitrogen and hydroxylation of the isopropyl group on the indole ring to provide additional active metabolites (Figure 10). None of these active metabolites circulates systemically, and they are not believed to contribute to the observed activity of this statin. However, there is the possibility of drug-drug interactions with other agents that are metabolized by, inhibit, or induce this isoform.

- Fluvastatin also inhibits CYP2C9. A potential drug-drug interaction with warfarin has been noted in the literature.

The free carboxylate group of all statins is vulnerable to Phase II glucuronidation.

Drugs or foods that inhibit the metabolism of the statins can increase the risk of serious adverse effects. Pravastatin and rosuvastatin, which are not metabolized by cytochromes to any appreciable extent, would be the safest statins in this regard. Fluvastatin, which is not metabolized by the CYP3A4 isoform, is next in line in the "safety parade." The CYP3A4-vulnerable statins would bring up the rear. Individuals with compromised liver function, and

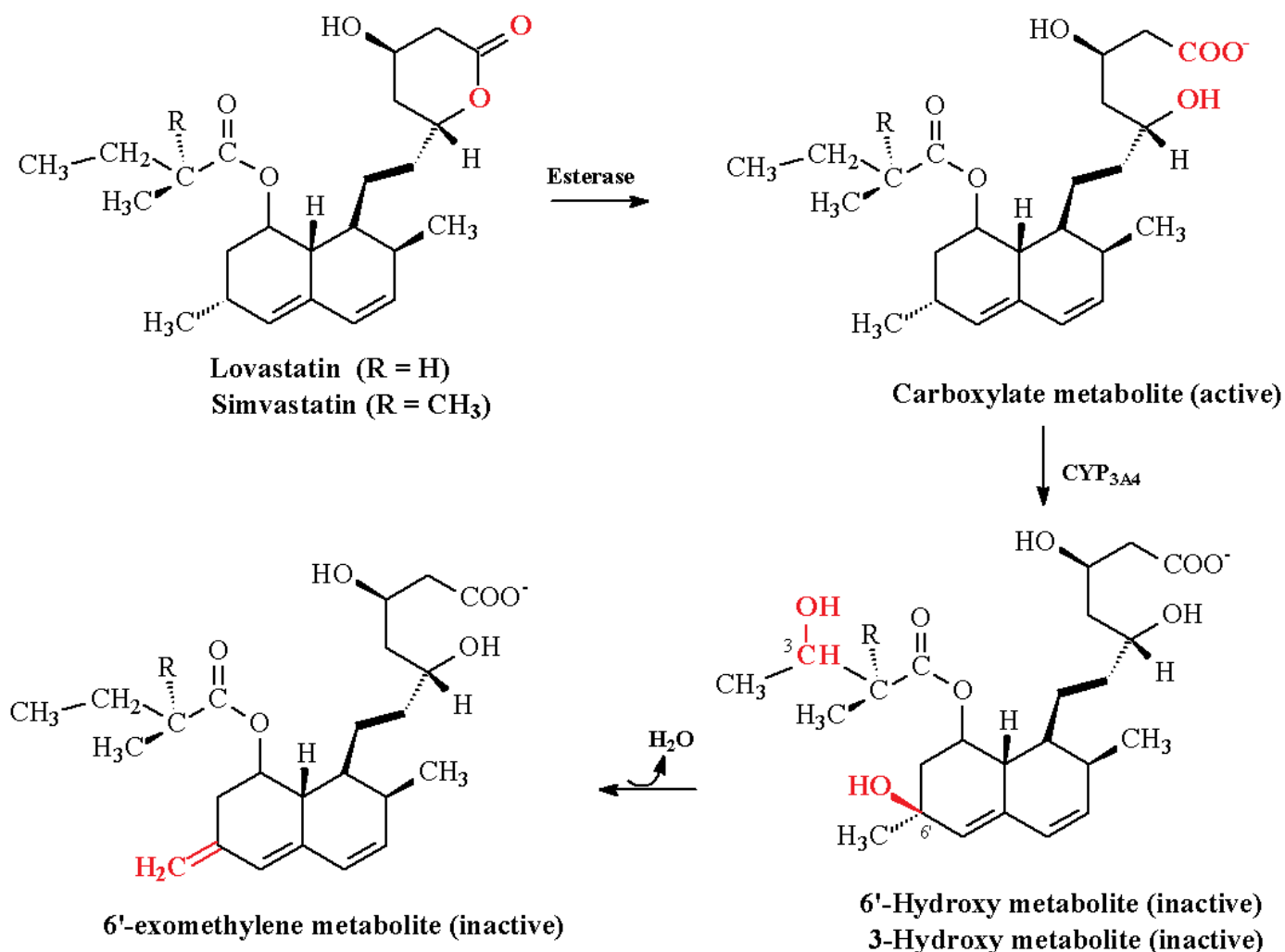


Figure 9. Lovastatin and simvastatin metabolism.

the elderly, are often at risk for use-limiting toxicity from CYP-vulnerable statins due to diminished inactivating metabolism. The risk of toxicity increases with the serum levels of active drug.

All statins except pravastatin are inhibitors of P-glycoprotein (P-gp), an ATP-dependent drug efflux pump. Many inhibitors of CYP metabolism also inhibit P-gp, and the enhanced risk of toxicity when statins are co-administered with CYP inhibitors may have as much to do with their competition for P-gp as for CYP isoforms.

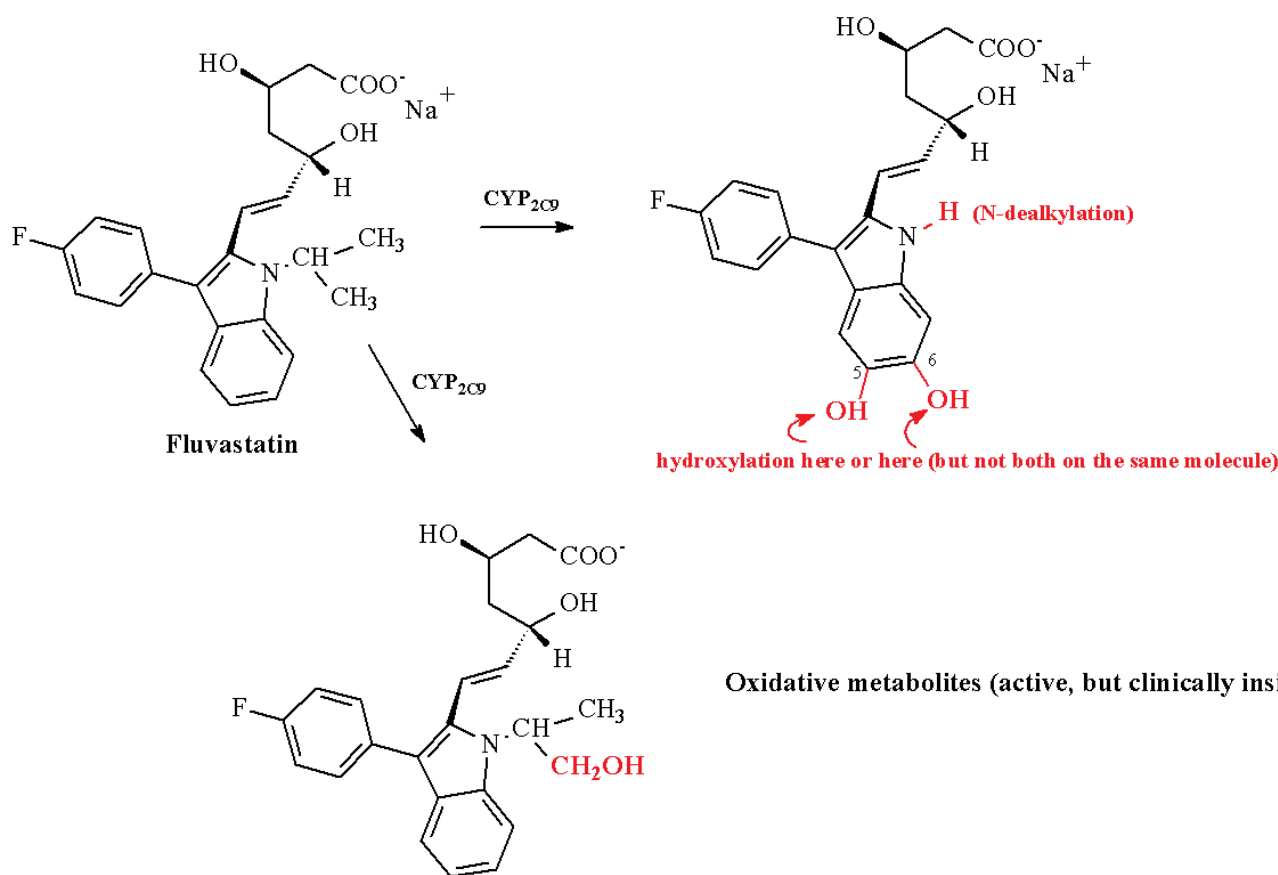
### Clinical Correlates

While not a metabolic interaction, oat bran and pectin inhibit the gastrointestinal absorption of all statins, and will decrease their effectiveness if consumed concurrently. Pectin is a polysaccharide found naturally in the "non-woody" components of fruits and vegetables, and is also used as a thickening agent in the making of jams and jellies. More importantly, perhaps, pectin is now being sold as a "nutraceutical" to lower serum cho-

lesterol, control appetite, and "aid digestion" (properties that appeal strongly to many US citizens). Apple pectin and grapefruit pectin products are being advertised on the Internet and sold in health food stores, and some *acidophilus lactobacillin* products (for lactose intolerance) have pectin added to their formulation.

Bile acid-sequestering agents contain quaternary ammonium groups and, if given together, they simply adsorb the anionic statins in the GI tract and block their oral absorption. Dual therapy with both statins and bile sequestering agents produces a greater reduction in total and LDL cholesterol than monotherapy; and successful co-therapy can be achieved as long as the statins are taken 1 hour before, or 4 hours after, the sequestering agents.

While GI distress is the most common complaint of individuals taking statins, the most serious adverse effects involve the liver (elevation of transaminase) and muscle (myalgia, myopathy, and a severe necrotic disorder called rhabdomyolysis, which often is accompanied by acute renal failure). The risk for rhabdomyolysis and other serious side



Oxidative metabolites (active, but clinically insignificant)

Figure 10. Fluvastatin metabolism.

effects is significantly increased if statins are co-administered with other lipid-lowering agents (eg, the fibrates, niacin), but there is currently controversy among health care providers about the wisdom of combination therapy.

**Fibrates.** The mechanism of interaction between the fibrates (especially gemfibrozil) and the statins is unknown, although one author suggests that the fibrates decrease the liver's ability to extract the statins, and therefore allow more statin to distribute to other tissues via the general circulation.<sup>14,30</sup> Some authors recommend that this combination be avoided, while others encourage its use because of the more comprehensive lipid-lowering action achieved with combination therapy. Statins have their highest impact of LDL and VLDL while the fibrates lower serum triglycerides and raise HDL levels.

**Niacin.** Despite the risk, low dose niacin complements the therapeutic utility of the statins. Like the fibrates, niacin significantly increases HDL cholesterol while also lowering triglycerides. Niacin also lowers LDL cholesterol. Combination therapy has been shown to increase "event-free survival" compared to statin monotherapy. Niacin's side effects are minimal when low doses (50 mg bid) are employed, and some statin/niacin combination products are now on the market (eg, Advicor).

The key to successful combination therapy with various lipid-lowering agents is to: (1) be aware of the potential for serious or life-threatening toxicity, (2) educate your patients on signs and symptoms to watch for, and (3) monitor vigilantly.

## REFERENCES

1. Roche VF. Utilizing chemical knowledge in rational therapeutic decision-making. *Am J Pharm Educ.* 1985;49:154-7.
2. Roche VF. The use of case studies in medicinal chemistry instruction. *Am J Pharm Educ.* 1993;57:436-9.
3. Roche VF, Alsharif NA. Stayin' alive: advancing medicinal chemistry by enhancing student responsibility for learning. *Am J Pharm Educ.* 2002;66:319-28.
4. Alsharif NZ, Theesen KA, Roche VF. Structurally-Based Therapeutic Evaluation: A therapeutic and practice approach to teaching medicinal chemistry. *Am J Pharm Educ.* 1997;61:55-60.
5. Alsharif NZ, Roche VF, Ogunbadeniya AM, Chapman RE. Evaluation of performance and learning parity between two required on-campus and Internet-based medicinal chemistry courses. *Am J Pharm Educ.* 2005;68:Article 33.
6. Roche VF, Zito SW. Computerized medicinal chemistry case studies. *Am J Pharm Educ.* 1997;61:447-52.
7. Roche VF, Aitken M, Zito SW. Evaluation of computerized medicinal chemistry case study modules as tools to enhance student learning and clinical problem-solving skills. *Am J Pharm Educ.* 1999;63:289-95.

8. Harrold M. Antihyperlipoproteinemics and inhibitors of cholesterol biosynthesis. In: Williams DA, Lemke TL, eds. *Foye's Principles of Medicinal Chemistry*. 5th ed. Baltimore, MD: Lippincott Williams & Wilkins;2002:580-603.
9. Istvan ES, Deisenhofer J. The structure of the catalytic portion of human HMG-CoA reductase. *Biochim Biophys Acta*. 2000;1529:9-18.
10. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*. 2001;292:1160-4.
11. Laufs U, Liao JK. Direct vascular effects of HMG-CoA reductase inhibitors. *Trends Cardiovasc Med*. 2000;19:143-8.
12. Knapp AC, Huang J, Starling G, Kiener PA. Inhibitors of HMG-CoA reductase sensitize human smooth muscle cells to Fas-ligand and cytokine-induced cell death. *Atherosclerosis*. 2000;152:217-27.
13. Kassbender K, Simons M, Bergmann C, et al. Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Ab42 and Ab40 in vitro and in vivo. *Proc Natl Acad Sci*. 2001;98:5856-61
14. Moghadasian MH. Clinical pharmacology of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Life Sci*. 1999;65:1329-37.
15. Evans M, Roberts A, Rees A. Pharmacological management of hyperlipidemia. *Br J Diabetes Vasc Dis*. 2003;3:204-10.
16. Blumenthal RS. Statins: effective antiatherosclerotic therapy. *Am Heart J*. 2000;139:577-83.
17. Gotto AM Jr. Statin therapy: Where are we? Where do we go next? *Am J Cardiol*. 2001;87:13B-18B.
18. Brown WV. Novel approaches to lipid lowering: What is on the horizon? *Am J Cardiol*. 2001;87:23B-27B.
19. McTaggart F, Buckett L, Davidson R., et al. Preclinical and clinical pharmacology of rosuvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. *Am J Cardiol*. 2001;87:28B-32B.
20. Olsson AG. Statin therapy and reduction in low-density lipoprotein cholesterol: Initial clinical data on the potent new statin rosuvastatin. *Am J Cardiol*. 2001;87:33B-36B.
21. McTaggart F. Comparative pharmacology of rosuvastatin. *Atheroscler Suppl*. 2003;4:9-14.
22. Garcia MJ, Reinoso RF, Sanchez-Navarro A, Prous JR. Clinical pharmacology of statins. *Meth Find Exp Clin Pharmacol*. 2003;25:455-79.
23. Hatanaka T. Clinical pharmacokinetics of pravastatin. *Drug Disposition*. 2000;39:397-412.
24. Worz CR, Böttorff M. Treating dyslipidemic patients with lipid-modifying and combination therapies. *Pharmacotherapy*. 2003;23:625-37.
25. Stein EA. The power of statins: aggressive lipid lowering. *Clin Cardiol*. 2003;4:25-31.
26. Ballantyne CM, Corsini A, Davidson MH, et al. Risk for myopathy with statin therapy in high-risk patients. *Arch Intern Med*. 2003;165:553-64.
27. Shek A, Ferrill MJ. Statin-fibrate combination therapy. *Ann Pharmacother*. 2001;35:908-17.
28. Portoghese PS, Sultana M, Nagase H, Takemori, AE. Application of the message-address concept in the design of highly potent and selective non-peptide delta opioid receptor antagonists. *J Med Chem*. 1988; 31:281-2.
29. Lipkowski AW, Tam SW, Portoghese PS. Peptides as receptor selectivity modulators of opiate pharmacophores. *J Med Chem*. 1986;29:1222-5.
30. Pierce LR, Wysowski DK, Gross TP. Myopathy and rhabdomyolysis associated with lovastatin-gemfibrozil combination therapy. *JAMA*. 1990;264:71-5.



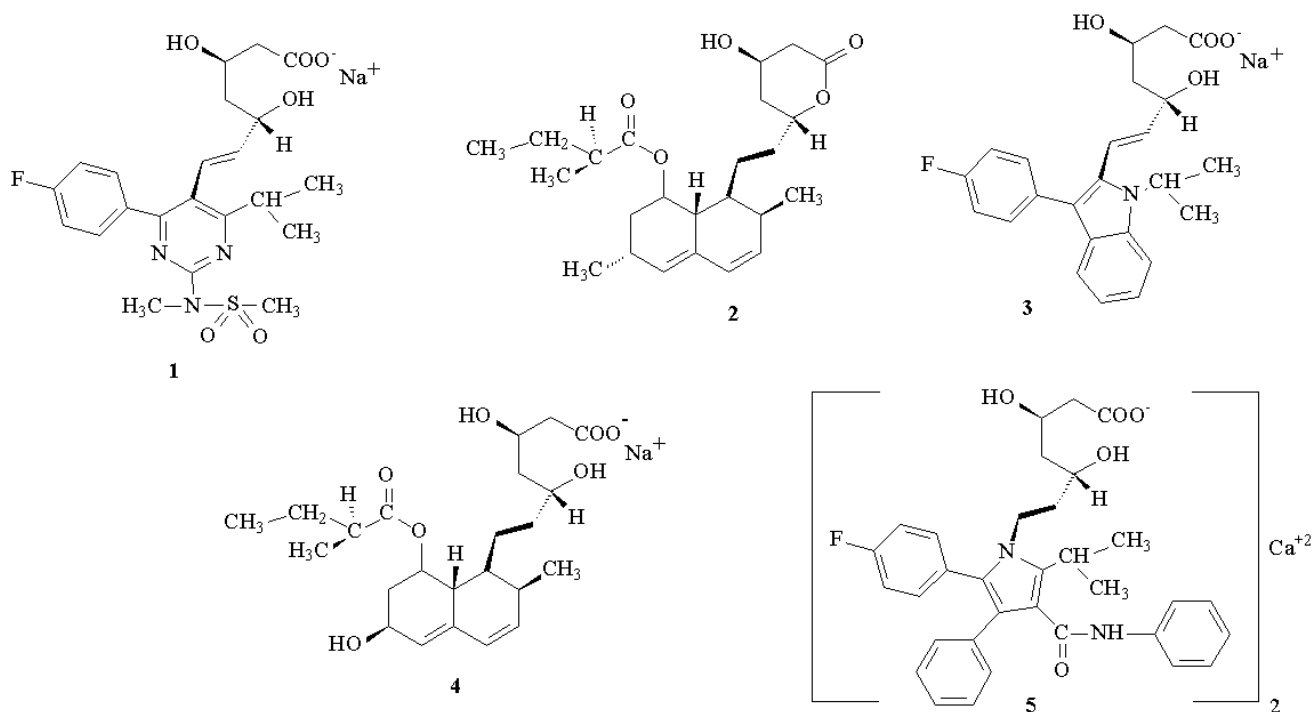
#### Appendix 1. Antihyperlipidemic Statins Learning Objectives

- Identify the important role of 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) in the biosynthesis of cholesterol, and why it is such an attractive target for cholesterol synthesis inhibitors.
- Discuss the structure of the HMGR enzyme, indicating where the substrate (HMG CoA) and the cofactor (NADPH) bind
- Identify the HMGR residues that bind the substrate (HMG CoA), and describe and name all important drug-receptor interactions.
- Compare and contrast the HMGR binding of HMG CoA and the statin antagonists, noting where they are the same, and where they differ. Recognize what component of the statins gives them access to the HMGR active site, and what component inactivates the receptor.
- Describe the conformational changes that occur in the HMGR receptor as a result of statin binding that increase affinity for the antagonists, and identify the pharmacological and therapeutic implications of this.
- Distinguish prodrug statins from active statins, explaining why the anionic form is required for HMGR inhibiting action, and how the active form is generated in vivo from the prodrug.
- Discuss the importance of the ring and sidechain substituents of the various statins on molecular lipophilicity, HMGR affinity, hepatic extraction ratio and degree of hepatoselectivity, protein binding, and risk of side effects (including CNS effects).
- Diagram the metabolic fate of all the statins that are metabolized by esterases and/or CYP450, identifying isoforms where known. Be able to draw the structures of all metabolites, and indicate whether they are active or inactive.
- From the excretion patterns of the marketed statins, predict the need for dosage adjustments in patients with hepatic and renal dysfunction.
- Identify potential drug-drug and drug-food interactions of the statins, and discuss the potential toxic consequences of these interactions.
- Identify the statins with long half-lives, and recognize the impact of this on the flexibility of time of drug administration (eg, hs vs. anytime).

#### Appendix 2. Study Questions for the “Antihyperlipidemic Statins: Chemistry and SAR” Lesson

1. What are the key residues involved in the binding of 3-hydroxy-3-methyl glutaric acid (HMG) to HMG CoA reductase (HMGR), and what bonds are formed when the two interact?
2. Which of the above reactions are mimicked by the statins? What drug-HMGR interactions are unique to the statins. What is the importance of the new interactions to the action of the statins?
3. Statin structures all contain a dihydroxyheptanoic acid section and a ring-containing segment. Which segment is responsible for providing access to the HMGR enzyme, and which segment is responsible for inhibiting it?
4. Why are lactone statins prodrugs? What enzyme and metabolic reaction release the active form?
5. What are the important CYP isoforms involved in the metabolism of selected statins? What are the pharmacological results of drug-drug or drug-food interactions that result in either competition for, or induction of, these isoforms?
6. Why is hepatoselectivity important in the action of the statins? What are the two ways by which statins can be transported into the liver (the site of action for these drugs)? What statin drug property is essential for each transport mechanism, and what functional group(s) on the statin structures provide them? What is the therapeutic benefit and risk of each mechanism of hepatic drug delivery?
7. What is the therapeutic benefit of statin action in the CNS? What physicochemical property is most critical in statin drugs that provide this benefit?
8. What foods and meds should be avoided at the time statins are taken, and why?

Appendix 3. Structure Challenge Exercise for the Antihyperlipidemic Statins Lessons



1. Which of these four statins can be given at any time of the day? What is the structural basis for your answer?
2. Which of these statins requires metabolic activation before it can inhibit HMG CoA reductase? Why is the parent structure inactive, and what metabolic reaction liberates the active form?
3. Which of these statins is/are the most hepatoselective? Why is hepatoselectivity desirable, and what is the chemical basis for the relative lack of extra-hepatic toxicity from the statin(s) you selected?
4. Which of these statins could be taken safely with grapefruit juice? What is the chemical basis for the lack of a drug-food interaction?
5. Which of these statins would have the highest affinity for the HMGR enzyme? What drug-enzyme interactions hold this statin to the binding site?
6. What is the chemical basis for the requirement to give statins one hour before or 4 hours after bile acid sequestering agents?