



HAL
open science

Statins in therapy: Cellular transport, side effects, drug-drug interactions and cytotoxicity -the unrecognized role of lactones

Clifford W Fong

► **To cite this version:**

Clifford W Fong. Statins in therapy: Cellular transport, side effects, drug-drug interactions and cytotoxicity -the unrecognized role of lactones. [Research Report] Eigenenergy, Adelaide, Australia. 2014. hal-01185910v1

HAL Id: hal-01185910

<https://hal.science/hal-01185910v1>

Submitted on 25 Aug 2015 (v1), last revised 2 Jul 2016 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Statins in therapy: Cellular transport, side effects, drug-drug interactions and cytotoxicity - the unrecognized role of lactones

Clifford W. Fong*

* Eigenenergy

Adelaide, South Australia, Australia

E mail: cwfong@internode.on.net

Keywords: Statin lactones, cellular transport, metabolism, drug interactions, quantitative models

Abstract

A molecular orbital computational study of the statins and a comprehensive review of the literature have found that the lactone forms of statins play a major role in the transport of statins across the cell membrane, and in the metabolism and clearance from the body. The neutral lactone and acid statin species are preferentially transported across the cell membrane and consequentially preferentially metabolised and cleared. The preferred cellular uptake of statin lactones has implications for cytotoxicity in muscle tissue and other side effects. The uptake mechanism is a combination of passive facilitated diffusion and active permeation by OATP transporters. Quantitative models describing how uptakes rates, binding affinity between statins and OATP transporters, and the inhibition of statin-OATP transporters processes are related to the four principal determinants of cell membrane permeability (desolvation, lipophilicity, dipole moment and molecular volume) have been deduced. Cyclosporin and gemfibrozil competitive inhibition of statin-CYP metabolism correlates with desolvation, lipophilicity, dipole moment and electron affinity, which suggests that electron affinity, a measure of reduction potential, is a useful indicator of potential drug-drug interactions (DDI). A similar relationship was found with the statin inhibition of paclitaxel oxidation by CYP enzymes. An examination of drugs known to cause DDI with statins show that reduction potential and cellular uptake properties are useful predictors of DDI. These statistical models, supported by literature evidence, indicate the statin lactones play a previously unrecognized major role in statin therapeutics, and in side effects, cytotoxicity and DDI.

Objectives:

- To understand the roles that statin acids and lactones play in statin therapeutics
- To investigate the side effects of statin therapeutics by understanding the transport of statins across cell membranes
- To investigate the metabolism and clearance of statins from the body
- To investigate the cytotoxicity and drug-drug interactions of statins
- To develop quantitative mechanistic models for statin transport and metabolic processes

1. Introduction

Statins are among the most widely prescribed drugs in the world. They are effective in lowering blood cholesterol levels, high levels of which can cause arteriosclerosis, and be detrimental to cardiovascular health. Statins can lower the risk of angina, heart attack and strokes. There can be other benefits other than cholesterol lowering effects, such as reducing the risk of esophageal cancer. While statins taken alone have shown remarkably few side

effects, it is clear that patients taking multiple drugs for other medical conditions can experience side effects and even toxic effects when there are drug-drug interactions (DDI). Statins are usually prescribed for people who have: atheroma-related diseases such as heart disease and atherosclerosis by reducing the chance that these conditions will worsen and can delay progression of the diseases, diabetes or another disease that increases the risk of developing an atheroma related disease, those with a family history of heart attacks, and the elderly.

Common side effects are headache, pins and needles, abdominal pain, bloating, diarrhoea, feeling sick, rashes and rarely, muscle inflammation. Statins may raise the risk of cataracts. The rare major side effects are liver failure (increased levels of liver enzymes), slightly increased risk of diabetes, and skeletal muscle damage, rhabdomyolysis, which can occur when statins are used in combination with other drugs that carry high rhabdomyolysis risk, or with other drugs that unintentionally raise the statin levels in the blood. People with liver disease, pregnant and breast feeding women, are not advised to take statins. Statins should not be combined with medications such as protease inhibitors (AIDS treatment), clarithromycin, erythromycin, itraconazole, clarithromycin, diltiazem, verapamil, grapefruit juice, niacin, or fibrate drugs (that also lower cholesterol LDL levels). Statins have not been found to increase the risk of cancer, and may actually reduce the risk of esophageal cancer, colorectal cancer, gastric cancer, hepatocellular carcinoma, and possibly prostate cancer. There is some evidence that the use of coenzyme Q10 supplements may help to prevent statin side effects in some people. [Mayo Clinic 2015]

The efficacy of drug therapeutics is governed by several critical factors, including:

- The administered dosage, when and how much, and what form of the drug reaches its target: statins are administered orally, and can be in acid, anionic-salt, or lactone form. The pK_a , binding to blood serum or human serum albumin (HSA), method of transport across the cell membrane, both uptake and efflux, and drug lipophilicity are commonly considered critical factors.
- How the drug is metabolised or cleared from the body. Bioavailability, half life, volume of distribution are key systemic factors, and metabolism, usually by the cytochrome P450 (CYP) enzyme family, can clear the parent drug, or can form metabolites which can also be active drugs.
- The potency of the statins is related to all of the above factors, as well as the actual binding energy of the statins to the HMG-CoA enzyme, which inhibits cholesterol formation.
- A summary of these factors is given in Table 1.

Clinicians categorize potential DDI with statins particularly by those that are metabolized by the hepatic isoenzyme CYP3A4, such as simvastatin and to a lesser extent atorvastatin, which are in combination contraindicated with drugs that *increase* systemic plasma levels of statins: (a) drugs that are potent inhibitors of CYP3A4 such as the macrolide antibiotics (clarithromycin, erythromycin), azole antifungals (itraconazole, ketoconazole), protease inhibitors (ritonavir), fibrates, cyclosporin etc; (b) other drugs that are moderate and lower inhibitors of CYP3A4, (c) drugs that induce CYP3A4 expression, and *reduce* statin bioavailability. Fluvastatin, pravastatin and rosuvastatin are not significantly metabolised by CYP3A4 and are clinically considered to be less susceptible to CYP interactions. [Medsafe 2014] Pravastatin, rosuvastatin, and pitavastatin are excreted mainly unchanged, and their plasma concentrations are not significantly increased by pure CYP3A4 inhibitors.

The role of uptake and efflux cell membrane transporters has recently been recognized as important in metabolic clearance of statins, particularly the inhibition of these transporters by certain drugs. For example, cyclosporin inhibits CYP3A4, P-glycoprotein (multidrug resistance protein 1 or MDRP1), organic anion transporting polypeptide 1B1 (OATP1B1), and some other hepatic uptake transporters. Gemfibrozil and its glucuronide inhibit CYP2C8 and OATP1B1. Many drugs are now known to inhibit OATP1B1 and related transporters. [Neuvonen 2010, Roth 2012, Kalliokioski 2009]

While metabolism may be a critical factor in statin DDI, it is clear that there are other factors that affect systemic clearance rates and bioavailability, particularly trans-membrane transport processes and the actual statins species that are involved in the plasma, cytosol, metabolic oxidation reactions etc. *This study will focus on the relevance of the anionic, acid and lactone form of the statins and their relative importance in transport and metabolic processes.*

2. Discussion

2.1 Acid and lactone forms of statins:

Statins exist in both acid and lactone forms in vivo. Statins can be administered as the acid form, the anionic-salt form, or for lovastatin and simvastatin, often as the lactone form. The lactones are converted in vivo by carboxyesterases in the intestinal wall, the liver and partially in plasma, a fully reversible reaction. It is also known that glucuronidation can convert the open acid form of the statins, whether administered as the acid or lactone, to the lactone form eventually, which is about 10% of the administered dose of statin. [Prueksaritanont 2002] The lactones have lower aqueous solubility than the acid forms, and are not necessarily better absorbed. A study of atorvastatin acid and lactone, and the metabolized products, 2- and 4-hydroxyatorvastatin, found that the acid compounds were stable in human serum at room temperature but the lactone compounds were unstable as they hydrolysed rapidly to their respective acid forms. The lactone compounds in serum could be stabilized by lowering the temperature to 4° C or lowering the serum pH to 6.0. Conversion of the acid form to lactones is H⁺ catalysed to drive the acid ⇌ lactone equilibrium towards the lactone side. [Jemal 1999] Atorvastatin undergoes pH-dependent hydroxy acid–lactone interconversion similar to other statins. Under both mildly acidic and basic conditions, the lactone form is less stable than its hydroxy acid form, but in the presence of a carboxylic acid, the equilibrium is slightly shifted towards the lactone side (4 kcal mol⁻¹ difference). [Hoffman 2008] The extracellular pH is usually about 0.5-0.6 higher than the intracellular pH, [Brandis] a factor which also favours the lactone form in the cytosol.

Both lactone and acid forms were observed in the systemic circulation after oral administration of atorvastatin [Kantola 1998a], lovastatin [Neuvonen 1996], simvastatin [Kantola 1998b, Prueksaritanont 2002], and cerivastatin [Backman 2002] in humans and/or animals, indicating that interconversion occurs between the lactone and acid forms of these statins. When atorvastatin was administered in the acid form to patients, the lactone form was found in almost equal quantities. [Kantola 1998a] The acid and lactone statins are also metabolized by the CYP enzymes, except pravastatin and rosuvastatin, with the lactones having a higher metabolic rate. [Fujino 2004, 2006] While it has been found that rosuvastatin is not significantly metabolized by the CYP enzymes, and is largely excreted unchanged, this situation is complicated by the finding that glucuronidation products of simvastatin acid, cerivastatin acid and rosuvastatin acid all undergo spontaneous cyclization to the lactone form. [Prueksaritanont 2002] Other statins (pitastatin, atorvastatin, fluvastatin) also undergo

the glucuronidation conversion to lactones. [Fujino 2004, 2006] These observations indicate that statins that cross the cell membrane, either in the acid or lactone form, and could be interconverted in the cytosol, and also could undergo glucuronidation in the mitochondria to the acyl conjugates, which can then spontaneously cyclise to the lactone form.

2.2 Transport of statins across the cellular membrane

Hepatocellular transport is a key facilitator of metabolism and clearance of drugs. Statin metabolism and elimination is largely dependent on how well the liver can uptake, metabolize and clear these drugs. OATP1B1 is almost exclusively expressed in hepatocytes where it is involved in the uptake of bile acids, eicosanoids, DHEA, estrogens etc. OATP1B1 is known to transport all statins in current use. A comparison of all statins showed that simvastatin pharmacokinetics were strongly influenced by SLCO1B1 521C>T (3.2fold increase in AUC) followed by atorvastatin, then either pravastatin or rosuvastatin. Fluvastatin AUC was not affected by SLCO1B1 521C>T because it is influxed into the liver by other transporters. Individuals with SLCO1B1 polymorphism have reduced rate of statin uptake resulting in slower metabolism and elimination. [Sissung 2012]

Studies of hepatocellular transport have shown that it is a competitive blend of passive non-saturable diffusion and active saturable transport using transporters. Passive diffusion is almost certainly a facilitated diffusion process in view of the large size of the statins where concentration gradients down a trans-membrane pores are involved. [Fong 2015]

2.3 Active transporters of statins:

The human OATP1B1-mediated transport of simvastatin acid, atorvastatin, pravastatin, pitavastatin, fluvastatin, rosuvastatin showed apparent K_m values between 0.6 to 29 μM , with atorvastatin having the highest and pravastatin the lowest affinity for OATP1B1. Simvastatin acid could not be determined due to its high passive permeability. [Sharma 2012] This data is consistent with the K_m values in Table 1 which indicates that the approximate binding energies of the statins with hepatocyte OATP transporters lies in the range -9.3 to -6.3 kcal/mol.

Corning TransportoCells OATP1B1 cells were used to characterize the intrinsic clearance rates for a series of statins, which showed a high correlation with the apparent hepatic uptake clearance rates in human hepatocytes. Pitavastatin, pravastatin, rosuvastatin, simvastatin, atorvastatin and fluvastatin showed K_m values of 0.2, 95.4, 14.2, 0.8, 0.2, 0.95 μM with corresponding hepatic clearance of 232.9, 11.5, 2.6, 37.9, 193.5, 85.7 L/mg/min. Contributions of OATP1B1 to the total hepatic uptake were 0.93, 0.82, 0.77, 0.69, 0.47, 0.18 respectively. The passive permeation rates (in control cells) for the statins were 20.7, 0.6, 0.2, 13.8 (simvastatin acid), 6.3, 31.4, plus cerivastatin 88.4 and lovastatin acid 50 $\mu\text{L}/\text{mg}/\text{min}$. [Li 2014] The passive permeation rates in the Corning TransportoCells correlated well with uptakes in human and rat hepatocytes. [Shitara 2006]

Atorvastatin uptake parameters for the OATP1B1, OATP1B3, OATP2B1 transporters were K_m 0.77, 0.73, 2.84 (μM) and V_{max} 6.61, 2.29, 24.27 (pmol/mg protein/min) respectively. Lovastatin acid, simvastatin acid and pravastatin acid were found to be specific OATP1B1 inhibitors. Pravastatin almost completely inhibits the uptake of atorvastatin by OATP1B1 (75%) with only a minor effect on OATP1B3 and OATP1B2 (15 and 5%) so overall atorvastatin uptake in the presence of pravastatin was reduced by 45%. [Karlgrén 2007]

The uptake of fluvastatin into rat cultured hepacytocytes has been shown to be a combination of non-specific diffusion and specific partly ATP driven partly Na^+ dependent saturable active transport. The two enantiomers of fluvastatin showed specific concentration and temperature dependent uptake processes, with K_m and V_{max} for the (+) and (-) isomers of 38.5 and 41.5 μM and 611 and 646 $\text{pmol}(\text{mg protein})^{-1}\text{min}^{-1}$ respectively. Simvastatin also shows a similar uptake mechanism to fluvastatin, being partly simple diffusion and specific active transport, whereas pravastatin K_m 32.3 μM shows only a specific OAT Na^+ independent mechanism with little passive diffusion in hepatocytes. Hepatocellular uptake of fluvastatin was inhibited by pravastatin, indicating a common transport system. In human aortic endothelial cells uptake of fluvastatin was by simple non-specific diffusion, via two binding sites with dissociation constants of 13.7 and 165 μM (calculated from the difference between uptake at 37° and 4° although it is possible that the 4° data may be partially due to reduced membrane fluidity). The drugs uptaken into the cells was shown to be predominantly the unchanged drugs. [Ohtawa 1999] The *estimated* binding free energy ΔG_{bind} for fluvastatin-OATP1B1 is -6.3 kcal/mol, and the value for the non-specific facilitated diffusion, presumably via a passive carrier protein, is about -6.9 kcal/mol at 37°C. Table 2 shows that the desolvation energy for fluvastatin anion, acid and lactone is 76.4, 25.6 and 14.2 kcal/mol respectively. The lipophilicities are -32.5, -17.9, -16.4, and the dipole moments are 36.6, 9.3 and 1.5 respectively. The molecular volumes are fairly similar. These data indicate that active transport by OATP1B1 or by a passive carrier protein are more likely to involve the neutral acid or more likely the neutral lactone forms.

Basolateral uptake of rosuvastatin in Caco-2 cells has a saturable and non-saturable component with an apparent K_m 4.2 μM , a saturable maximum flux J_{max} 6.1 $\text{pmol}\cdot\text{min}^{-1}\cdot\text{mgprotein}^{-1}$, and a facilitated diffusion coefficient 1.0 $\mu\text{l}\cdot\text{min}^{-1}\cdot\text{mgprotein}^{-1}$. [Li 2012]

The uptake of rosuvastatin by OATP1B1 in hepatocytes is strongly inhibited by gemfibrozil IC_{50} 4.0 μM and cyclosporin 2.2 μM . In healthy people, this led to an average exposure increase to rosuvastatin of 1.9 fold for gemfibrozil, and a very large 7.1 fold total higher exposure for cyclosporin. Other statins show large AUC fold increases when co-administered with gemfibrozil or cyclosporin [Schneck 2004, Simonson 2004, Kalliokioska 2009] It is unclear which species of statin were transported in the inhibition studies. There is now a 2012 FDA and European Medicines Agency requirement to consider if an investigational drug is a substrate of OATP1B1 or OATP1B3 during clinical trials, particularly if hepatic elimination is > 25%. The current medication guide for Crestor, rosuvastatin, warns that co-administration medications such as cyclosporin which are inhibitors of OATP1B1 transporters, may lead to increased plasma levels of rosuvastatin and increased risk of myopathy.

2.4 CYP metabolism:

As shown in Table 1, all statins undergo CYP3A4 metabolism with the exception of pravastatin, pitastatin and rosuvastatin, and fluvastatin undergoes CYP2C9 metabolism. Due to rapid metabolism in the gut and liver, the system bioavailability is generally low, except for cerivastatin and pitastatin.

The statin acids and particularly the lactones are considered to have high first pass metabolism, but the situation is complicated in blood plasma by the high binding to human serum albumin (HSA) for all statins, except pravastatin. The bioavailability of the statins (see

Table 1) is complicated by the pH dependent equilibrium between acid and lactone forms, and the HSA binding. It is safe to conclude that it is unclear what are the active species for each statin, particularly when categorized into active species in blood serum, the species being transported across the cell membrane, both uptake and efflux, and the species being metabolized by CYP enzymes intracellularly, and the relationship between first pass metabolism and bioavailability. Where a DDI can occur, or where pre-existing medical conditions exist, eg diabetes, kidney transplants etc, the situation becomes even more complex. The more bioavailable the statin form, the more the higher the systemic concentration, and the more likely a DDI can occur.

The metabolic clearances of the acid and lactone forms of atorvastatin, simvastatin, cerivastatin, fluvastatin, rosuvastatin and pitastatin from human hepatic microsomes at 2.5 μM are 26, 28, 21, 33, 1, 3 and 1892, 1959, 622, 226, 71, 5 ($\mu\text{L}/\text{min}/\text{mg}$ protein) respectively. [Fujino 2004, 2006] The CL_l lactone/acid clearance ratios are 73, 70, 30, 7, 71, 2 respectively. Metabolic clearance is predominantly driven by cellular transport and CYP oxidation, and the acid and lactone metabolism was dependant on CYP3A4 with the exceptions of CYP2C9 being the dominant enzyme for fluvastatin and pitastatin, and CYP2C8 and CYP3A4 being of equal importance for cerivastatin, and rosuvastatin acid being mostly excreted unchanged. *The CYP3A4-mediated metabolism of the lactone forms is a common metabolic pathway for statins.* The metabolic oxidation by the CYP enzymes leads to metabolites which are effluxed out of the cell and excreted. The situation is further complicated by the observation that CYP metabolites of lactones can hydrolyse to acids. Some drugs are known to competitively bind to these CYP enzymes when present with statins, which have a narrow therapeutic index, leading to reduced oxidative metabolism of the statins, and increasing statins concentrations with concomitant side effects.

The low CL_l lactone/acid clearance ratio for pitastatin, 2, is related to the fact that it is only slightly metabolized by the CYP2C9 enzyme, hence its high bioavailability of 60%. Pitavastatin is minimally metabolized and most of the bioavailable fraction of an oral dose is excreted unchanged in the bile and is reabsorbed by the small intestine ready for enterohepatic recirculation. Its metabolite is the lactone. Neither pitavastatin nor its lactone form, have inhibitory effects on CYP enzymes, and CYP3A4 inhibitors have no effect on pitavastatin concentrations. Moreover, P-glycoprotein-mediated transport does not play a major role and pitavastatin does not inhibit P-glycoprotein activity. Pitavastatin is transported into the liver by several hepatic transporters but OATP1B1 inhibitors have relatively little effect on plasma concentrations compared with other statins. Pitavastatin has minimal drug-food and drug-drug interactions making it a treatment option in the large group of dyslipidaemic people who require multidrug therapy. Pitastatin has significant LDL-C-lowering efficacy at low doses compared to other statins. [Catapano 2010, Saito 2011].

Uptake (solute carriers, SLC families) and efflux (ATP binding cassette, ABC families) transporters are major determinants of statin therapeutics, and along with the efficacy of metabolic disposal by CYP enzymes, govern the therapeutic index of these drugs. [Rodrigues 2010, Niemi 2010, Roth 2011] As previously discussed in section 2.4 and 2.6 the cellular transport of the statins is a competitive process between passive facilitated diffusion and active transport. However a critical factor which pre-determines the uptake of statins from the blood is the known reversible binding interaction of statins with blood proteins, particularly human serum albumin (HSA). HSA is the principal carrier of unesterified free fatty acids in serum and its major functions include maintaining osmotic pressure, transporting endogenous and exogenous ligands. At physiological pH levels, HSA is negatively charged. It is known

that all the approved statins in clinical use are highly bound to HSA (> 90%) with the exception of pravastatin which is ca. 50% bound (Table 1). Since all statins, except lovastatin and simvastatin, which can be given in the lactone form (but then bio-transformed to the acid forms), are administered in the acid form (rosuvastatin, atorvastatin, fluvastatin, simvastatin can also be given in the anionic salt forms) which transform to the acid form at physiological pH), it can be assumed that the acid forms are the species reversibly binding to HSA. As it is known that statin acids can be in equilibrium with the lactone form in blood serum, it is likely that either neutral species could be the transported species that crosses the cell membrane by passive or active transport. [Skottheim 2008, Hoffman 2008, Sakaeda 2005, Chen 2005, 2007, Narwal 2010]

In a study of the acid and lactone forms of atorvastatin, lovastatin and simvastatin (and pravastatin in acid form), it was found that the **IC₅₀ values** (transfected canine kidney cells) for the **uptake transporter OATP1B1 were 3-7 times lower for the acid forms than the lactone forms**, but for the **efflux transporters MDR1, MRP2, Mrp2, the IC₅₀ values for the lactone forms were up to 10 times lower**. [Chen 2004] Bidirectional transport studies of atorvastatin, fluvastatin, rosuvastatin, lovastatin and simvastatin in Caco-2 cell monolayers showed that atorvastatin, fluvastatin and rosuvastatin acids had much higher basolateral to apical than apical to basolateral rates. However the lovastatin and simvastatin lactones showed little efflux, and a substantial amount of the lactone was converted into the acid form, but there was little efflux of the newly created acid form. [Volpe 2010] Oral administration of atorvastatin, lovastatin and simvastatin in acid form in humans has been shown to produce both acid and lactone forms in plasma, which is opposite from that shown in mice after subcutaneous injection. [Chen 2007]

In a study of the CYP metabolism and DDI interactions of the acid and lactone forms of atorvastatin, it was found that the lactone pathway is the major determinant of metabolic clearance and DDI. Metabolism by CYP3A4/5 of both statins resulted in para- and ortho-hydroxy metabolites, and the lactone had a higher affinity to CYP3A4 than the acid form, with the K_m values for para-hydroxyatorvastatin 1.4 and 25.6, and for ortho-hydroxyatorvastatin 3.9 and 29.7 μM respectively. It is noted that the binding affinity of the lactone is more than 5 times those for lovastatin and simvastatin. CYP dependent metabolism of the lactone was 83 fold higher than for the acid (para-hydroxylation), and was 20 fold higher for ortho-hydroxylation. Atorvastatin lactone was a potent inhibitor of the metabolism of the acid by human liver microsomes, K_i 0.9 μM, but the atorvastatin acid was a poor inhibitor of the metabolism of the lactone, K_i 90 μM. The experimental binding energies for the lactone and acid in CYP3A4 were 1.3 and 1.8 kcal/mol for ortho- and para-hydroxylation. [Jacobson 2000] It has been shown that DDI between lovastatin and simvastatin (administered as the lactone) and itraconazole led to a > 20 fold and 18.8 fold increase in the area under the curve AUC values, whereas the same DDI with atorvastatin led to a 3.2 fold increase in AUC. [Kantola 1998] This observation is consistent with the higher binding affinity of atorvastatin compared to those for lovastatin and simvastatin

High plasma levels of the lactone forms have been observed in patients with statin induced myopathy. In primary human skeletal muscle cells, atorvastatin lactone showed a 14-fold, fluvastatin lactone a 26-fold, pravastatin lactone a 23-fold, and simvastatin lactone a 37-fold higher potency to induce myotoxicity compared to their corresponding acid forms. [Skottheim 2008]

In a study of the OATP1B1 hepatic transporter inhibitor, rifampin, on the kinetics of atorvastatin and its metabolites in humans. An intravenous dose of rifampin significantly increased the plasma concentration of atorvastatin acid by 6.8 fold and that of the metabolites 2-hydroxy-atorvastatin acid and 4-hydroxy-atorvastatin acid by 6.8 and 3.9 fold, respectively. The levels of the lactone forms of atorvastatin, 2-hydroxy-atorvastatin and the metabolite 4-hydroxy-atorvastatin, were also significantly increased, but to a lower extent. The data confirm that OATP1B transporters represent the major hepatic uptake systems for atorvastatin and its active metabolites. [Lau 2007]

Gemfibrozil can modulate the pharmacokinetics of statins more via inhibition of statin hydroxyl acid glucuronidation than via inhibition of CYP3A4-mediated oxidation. Gemfibrozil is not a strong inhibitor of CYP3A4. Glucuronidation is a common, metabolic pathway for the conversion of active open acid forms of several statins (including atorvastatin and rosuvastatin) to their lactone form. The lactone form, in turn, plays a critical role in the subsequent statin metabolism catalyzed by CYP3A4. Consistent with the severe interactions reported with gemfibrozil, cerivastatin was shown to be more susceptible than simvastatin and atorvastatin to metabolic glucuronidation with gemfibrozil. [Bellostra 2004]

Gemfibrozil co-administration with statins results in AUC increases for simvastatin, lovastatin, pravastatin (all in the 2-3 fold range), cerivastatin 5.6 fold, and atorvastatin about 1.3 fold. The glucuronidation of atorvastatin yields an acyl glucuronide which undergoes pH dependent lactonization to atorvastatin lactone, K_m 12 μ M, K_i 75 μ M. Atorvastatin lactone gives an ether glucuronide, K_m 2.6 μ M. Gemfibrozil, fenofibrate, and fenofibric acid inhibited atorvastatin lactonization with IC_{50} values of 346, 320, and 291 μ M respectively. Based on unbound fibrate concentrations at the inlet to the liver, these data predict a small increase in atorvastatin AUC (1.2 fold) after gemfibrozil coadministration and no interaction with fenofibrate. [Goosen 2007]

2.5 Effect of statins on patients with pre-existing medical conditions:

In a study of the effect of atorvastatin acid and lactone in patients with (and without) diabetes mellitus, the effect of diabetes on the biotransformation of atorvastatin acid, both in vivo in nondiabetic and diabetic renal transplant recipients, and in vitro in human liver samples from nondiabetic and diabetic donors was examined. In diabetic patients, the plasma concentration of atorvastatin lactone was significantly higher than that of atorvastatin acid throughout the 24-hour sampling period. Diabetic patients have 3.6 times lower apparent total clearance than nondiabetic patients. The concentration of atorvastatin acid remaining in the microsomal incubation was not significantly different between nondiabetic and diabetic liver samples, whereas the concentration of atorvastatin lactone was significantly higher in the samples from diabetic donors. CYP3A4 was responsible for the biotransformation of atorvastatin lactone. [Dostalek 2012]

In a similar study of the effects of atorvastatin acid and lactone in patients with the kidney transplants demonstrated a significant reduction of clearance of atorvastatin lactone compared to the acid form, and thus they may be at higher risk of developing myotoxicity, since atorvastatin lactone is known to increase myotoxicity. [Mewan 2013]

2.6 Clearance and elimination of statins:

The metabolic clearance rate is the volume of biological fluid completely cleared of drug metabolites as measured in unit time. Elimination occurs as a result of metabolic processes in the kidney, liver, saliva, sweat, intestine, heart, brain, or other sites. Because statins are highly bound to blood serum proteins, clearance is the elimination of (free statins + plasma protein bound statins). The clearance rate for protein bound statins is lowered by protein binding, which is >90% for all statins except pravastatin at ca. 50% (Table 1). Clearance in the kidney is mainly by filtration, whereas in other sites, clearance is by membrane transport proteins. Clearance in the liver is mainly by filtration through the sinusoidal system.

It has been shown that the rate determining process in the hepatic elimination of statins (pravastatin, pitastatin, atorvastatin and fluvastatin) in rats and humans is the uptake process. This finding using cryopreserved human hepatocytes and a rat scaling in vitro factor (based on the finding that scaling factors for P450-mediated metabolism are preserved in humans and rats). The in vivo uptake clearances were pravastatin 1.44, pitavastatin 30.6, atorvastatin 12.7, and fluvastatin 62.9 (ml/min/g liver), compared to clinically determined values for intrinsic clearance of 0.8-1.2, 14-35, 11-19, and 123-185 respectively. The OATP transporters were the saturable uptake agents. [Watanabe 2010] These results are similar to those observed in Corning Transportocells OATP1B1 where a linear relationship between the intrinsic uptake clearance for the statins and clinically observed hepatic uptake clearances was observed. [Li 2014]

Table 3 shows the metabolic clearance for the acid and lactone statins forms from human hepatic microsomes. The very high lactone:acid clearance ratio for all statins (except pitastatin at 2) reinforces the various studies described in section 2.4 and 2.6.

2.7 Cytotoxicity of statins:

The cytotoxicity of statins ranges from their use as anti-cancer agents [Kunzl 2013, Gazzero 2012] to extreme side effects, particularly cardiomyopathy. The withdrawal of cerivastatin from the market is a well known example. Since statins inhibit cholesterol synthesis mainly in the liver, it is not surprising that elevations in the liver enzymes ALT and AST can occur. The principal safety issue with statins is the potential to cause acute liver failure. Four types of hepatic syndromes need to be considered: acute liver failure, hepatitis, cholestasis and transaminitis.

The cardiac effects of statins are subject to controversy, and the mechanism of their uptake into the human heart is unknown. The expression of OATP2B1 was analyzed in 46 human atrial and 15 ventricular samples, including samples from hearts with dilated cardiomyopathy and hearts with ischemic cardiomyopathy. OATP2B1 is a high-affinity uptake transporter for atorvastatin (K_m 0.2 μ M) and is expressed in the vascular endothelium of the human heart, suggesting its involvement in cardiac uptake of atorvastatin. Simvastatin (lactone) was not transported by OATP2B1. Patients who had taken atorvastatin exhibit decreased OATP2B1 messenger ribonucleic acid expression compared with patients with no statin treatment. [Grube 2006]

In primary human skeletal muscle cells, atorvastatin lactone showed a 14-fold, fluvastatin lactone a 26-fold, pravastatin lactone a 23-fold, and simvastatin lactone a 37-fold higher potency to induce myotoxicity compared to their corresponding acid forms. [Skottheim 2008]

In a study of the relationship between pravastatin- and rosuvastatin-induced cytotoxicity and medium pH using human prototypic embryonal rhabdomyosarcoma cell line (RD) and rat myoblast cell line (L6) as a model of in vitro skeletal muscle, it was found that rosuvastatin cytotoxicity, reduction of cell viability, morphological changes and caspase activation at acidic pH (pH 6.8) were significantly greater than those at neutral pH (pH 7.4). Rosuvastatin accumulation at acidic pH was greater than that at pH 7.4. Medium pH had no effect on pravastatin accumulation or cytotoxicity. Rosuvastatin cytotoxicity at acidic pH is associated with increasing intracellular accumulation of rosuvastatin. [Kobayashi 2007]

A study of simvastatin, lovastatin and pravastatin induced cytotoxicity on B16.F10 murine melanoma cells in vitro has shown that only simvastatin and lovastatin exhibited cytotoxicity. The cytotoxic actions were mainly based on the suppressive actions on hypoxia-inducible factor 1 α expression and nonenzymatic antioxidant levels, as well as because of the inhibition of superoxide dismutase activity in B16.F10 melanoma cells. [Alupej 2014]

3. Results

3.1 Energetics of cellular membrane transport processes:

To gain an understanding of the differences between the passive diffusion and active transport mechanisms, an understanding of the relative energies involved is required. Passive diffusion, including facilitated diffusion (which uses a trans-membrane spanning protein) does not require any external energy source to drive the transport, whereas active transport which uses a transporter to move a substrate against the electrochemical gradient requires an energy source, usually ATP. The free energy of ATP hydrolysis ΔG_{ATP} is about 14.3 kcal/mol under cellular conditions. Transport systems can use the energy contained in trans-membrane gradients to add to the energy from ATP or GTP cycles. The total electrochemical potential gradient averages about -170 mV for normal cell membranes. [Nicholls 1992] The cell membrane potential is about -40 to -80 mV (about 1-2 kcal/mol). The transport of drugs by active transporters requires considerable energy. For example, the energy expended by cells to maintain the concentration gradients of Na⁺, K⁺, H⁺, and Ca²⁺ across the plasma and intracellular membranes in nerve and kidney cells, requires about 25 percent of the ATP produced by the cell for ion transport; and in human erythrocytes, up to 50 percent of the available ATP is used for this purpose. [Lodish 2000] Cations are known to generally more easily be transported across the cell membrane than anions. Little is known about the free energy required to transport large neutral and charged molecules (drugs, proteins etc) across cell membranes. The transport of small bare neutral and charged molecular species across the membrane electrical gradient can in principle be calculated using the Nernst equations, but other factors such as desolvation, binding energy between the substrate and transporter, and conformational changes required for larger molecules and/or the protein transporters during binding can have very large energy penalties, which would require many ATP or GTP energy releases to drive active transporters. [Fong 2015, Alder 2003, Lodish 2004] The experimental binding energies between substrates and transporters are roughly in the region of -8 to -20 kcal/mol (for instance the binding energy of the aspartate transporter Glt_{ph} (Pyrococcus horikoshii), is -12 kcal/mol Boudker 2007), while the activation energy for the temperature dependent transport reaction of GlpT-G3P phosphate in E.coli is 8.4 kcal/mol. [Law 2007] Typical antibody-antigen dissociation constants are characteristically near 10⁻⁸ M (free energy of binding is -11 kcal/mol), ATP binds to myosin with a dissociation constant of 10⁻¹³ M (free energy of binding is -17.9 kcal/mol) and biotin binds (effectively irreversibly) to avidin, a protein found in egg white, with a dissociation constant of 10⁻¹⁵ M (free energy of

binding is -20.6 kcal/mol), which is the strongest known (non-covalent) small molecule-protein binding energy. [Kuriyan 2009, Chaplin 1990] The binding energies of statins with HMG-CoA reductase are in the region of -9 to -12.5 kcal/mol. [Freire 2008] The loss of translational and rotation entropy of the small molecule ligands on binding can contribute a penalty up to about 12 kcal/mol towards the total binding energy. The bound conformation of the ligand is usually higher than the free ligand, by about an average of 4-5 kcal/mol, with 10% of ligands having a higher energy of up to 9 kcal/mol. [Perola 2004]

To get an understanding of the magnitude of free energy changes involved in passive and active transport, an examination of the known uptake mechanism of D- and L-glucose by human red blood cells is instructive. The facilitated diffusion of D- and L-glucose by the Glut1 transporter is highly stereospecific with the D-isomer having a K_m of 1.5 mM, compared to the L-isomer value of >3000 mM. This corresponds to a binding free energy difference of greater than -4.7 kcal/mol. There is a single trans-membrane spanning protein with a single site, accessible from inside or outside of the membrane, and the glucose causes a conformational change of the protein transporter. [Kuypers 2008] If the concentration of D-glucose inside the cell was say 0.05 mM, and the extracellular concentration was say 5.0 mM, then at 310K the free energy ($\Delta G = RT \ln[\text{intra-cellular}]/[\text{extra-cellular concentration}]$) of *non-saturable facilitated transport* would be about -2.9 kcal/mol. However if *active saturable transport* was required to say translocate D-glucose against the concentration where the extracellular concentration was say 0.005 mM and the intracellular concentration was say 5.0 mM, then the ΔG required is about +4.3 kcal/mol. It is known that the active transport of glucose requires two Na^+ to facilitate active transport: if the extracellular and intracellular concentrations of Na^+ were say 140 and 10 mM (typical values for mammalian cells), then the ΔG available is 2×-1.6 kcal/mol, roughly -3.2 kcal/mol. The electrical gradient across the membrane can be calculated from the Nernst equation ($\Delta G = z.F.V_m$ where z is the ionic charge, F is the energy released as one mole of charge moves down a voltage gradient, and V_m is cellular membrane potential, about 70mV for mammalian cells. For two Na^+ this translates to roughly -3.2 kcal/mol, so the total *electrochemical gradient* to move glucose against a steep concentration gradient (ie from 0.005 outside to 5.0 mM inside) is about -3.2 plus -3.2 kcal/mol which is sufficient to overcome the +4.3 kcal/mol required. The Na^+ gradient across the plasma membrane is created by the active transport of Na^+ out of the cell by the Na^+/K^+ ATPase pump.

So from Table 2, it can be seen that desolvation energies of substrates prior to binding with active transporters, and the biophysical properties of the ligand species being transported, particularly the lipophilicity, dipole moment, and molecular size, can be comparable in magnitude to cellular transport energies and transporter binding energies, without even considering transporter protein conformational energy changes. The desolvation data for the anions, acids or lactones has strong implications for what species are actually being transported, since the penalty for the anionic species are very high compared to the neutral species. For example, in Table 2 it can be seen that the desolvation energies for the anionic statin species range from 74-104 kcal/mol, while the acid species range from 25-38 kcal/mol, while the lactones range from 14-32 kcal/mol. The difference between the neutral species and the anionic species is very large, considering the range of energies involved in transport processes discussed in the above two paragraphs. While it is unknown what degree of desolvation is required for active and passive transcellular processes, the desolvation energy gap is simply too large for charged species. In addition, substantially desolvated charged species have a strong electrostatic effect, which can be repelled by hydrophobic protein surfaces, but possibly attracted to protein sites of the opposite charge. The dipole moment is a

measure of electrostatic polarity, Since these neutral and charged species are in equilibrium in blood plasma, *it is likely that the neutral species, most probably the lactones, are the actual species being preferentially translocated by the OATP transporters*, rather than the anionic species.

3.2 Redox metabolism by CYP enzymes

The metabolic transformation of drugs, including the statins, in the mitochondria can be found experimentally *in vitro*. The oxidation-reduction mechanism is well known, involving electron transfer from a heme centred moiety within the CYP enzyme to the substrate. A useful theoretical means of predicting this redox mechanism is the reduction potential of the substrate. The electron affinity (EA) is a measure of the acceptance of an electron by a molecule, and so the EA is a measure to the ease of reduction (acceptance of an electron) to form metabolites such as hydroxylated species etc. [Fong 2014]. Table 2 shows the calculated EA for the various statin anion, acid and lactone species in water. It can be seen that there are some very significant differences for the same statin when in the anion, acid, and lactone forms. The use of EA as an indicator of ease of reduction is only applicable to a series of substrates which have a strong chemical structural similarity, and strong linear relationships have been observed between reduction potentials and substrate reactivity properties. These relationships appear to be present in the three statin forms in Table 2, with the one exception of rosuvastatin lactone where the EA value is abnormally higher than the other statin lactones, and appears to be related to the lower charges on the atoms of the (unique to the statins) methanesulphonate group. This value was omitted from development of the statistical models described below.

The electrochemical redox properties of the statins have been determined in a number of studies. Generally, with the exception of pravastatin, which can show reversible redox behaviour, the statins undergo irreversible reduction in acid and alkaline buffers, and often show linear relationships between peak reduction potentials and pH. Irreversible redox behaviour is a result of the formation of reduced species, and for simvastatin and lovastatin lactones, probably being the formation of the acid species at acid pH, and for other statins, tested as the anionic salts, probably hydroxylation. The acid form of the anionic salt would predominate at acid pH levels. The statin lactones are known to be H⁺ catalytically hydrolysed at acid pH in blood serum to the acid form, but the equilibrium can be pushed to the lactone side at pH 6. [Jemal 1999] However the acid:lactone equilibrium may be different in the absence of serum proteins, as in the electrochemical studies, where it was observed that the peak reduction potential of simvastatin was independent of pH above pH 5 (up to pH 11), [Coruh 2006] which probably suggests that it is the acid form of simvastatin that predominates above pH 5 to 7, and the anionic form at > pH 7. The following peak reduction potentials (relative to Ag/AgCl reference electrode) at ~pH 5 were simvastatin lactone 1.12V [Coruh 2006], lovastatin lactone 1.49V pH 6 [Nigovic, Pakovic 2009], fluvastatin 0.64V [Yan 2006], rosuvastatin 1.18V [Altinoz 2013], pravastatin 1.32V [Nikovoc 2009], atorvastatin 0.92V [Dogan-Topal 2009], pitastatin 1.21V pH 6. [Janagiraman] The absolute reduction potential were calculated according to the method of Topol 2001 to compare with the experimental electrochemical reduction potentials and the EA values calculated in water. It was found that the trends were similar in all cases, but exceptions were outliers from the general trend, notably rosuvastatin lactone which has an anomalously high EA. The electrochemical values for fluvastatin and pravastatin acid seemed much lower than the other experimental values, which reflect the chemical equilibria at ~pH 5.

The biophysical data of a number of drugs that cause DDI with statins are listed in Table 2. It can be seen that the neutral species of these drugs have similar properties as the neutral acid or lactone forms of the statins, so easily fit into the therapeutic window for these drugs. It is suggested that such a criteria is a useful screening tool for possible DDI with statins. The very high desolvation energies for the charged species would effectively preclude these forms of the drugs from being active in transport processes, and hence any interaction with CYP enzymes. Comparison of the fibrates, gemfibrozil, fenofibrate, and fenofibric acid shows that they have similar properties with the exception of their EA values, 0.6, 2.6, 2.4 eV respectively, which indicates that the species would have similar cell transport properties, but the neutral form of gemfibrozil would passively and actively transport much faster. The higher EA for fenofibrate and fenofibric acid means that these drugs would be reduced faster than gemfibrozil by glucuronidase or CYP enzymes, and thus less likely to inhibit the enzymes. This data is consistent with the observed clinical effects where gemfibrozil can cause serious DDI with statins, but fenofibrate does not. [Bellostra 2004, Goosen 2007] Gemfibrozil and its glucuronide inhibit CYP2C8 and OATP1B1. [Neuvonen 2010, Roth 2012, Kalliokioski 2009] Gemfibrozil has also been shown to inhibit the OATP1B1 uptake of rosuvastatin [Schneck 2004]

Statin DDI involving warfarin, niacin anions, verpamil or mifefradil are more likely to occur with the neutral species rather than their co-equilibrated ionic species based on the very large desolvation penalties required for the ionic species to interact with transporters of CYP enzymes. Cyclosporin and digoxin are unusual in that these neutral drugs have very large desolvation values, which may indicate that their main effect is to inhibit statin transport. Digoxin is a substrate of OATP1B3 or possibly another transporter [Oswald 2012, Kimoto 2011] and cyclosporin is reported to passively diffuse (presumably facilitated diffusion in view of the large molecular size of cyclosporin) into rat hepatocytes and has a membrane fluidising effect. [Fricker 1997]

It is noted that coenzyme Q10, ubiquinone, fits into this therapeutic window, and its high EA would suggest that it would be effective in mitigating any redox processes such as reactive oxygen species (ROS) that could be involved in cytotoxic oxidative stress side reactions. Statins are known to decrease the cellular levels of glutathione, and increase the levels of oxidised glutathione, an indicator of oxidative stress. [Eghbal 2014] This observation gives some basis to the moderate use of coenzyme Q10 supplements as being useful in offsetting statin side effects such as myalgia. [Mayo clinic 2015]

Endothelial dysfunction and the imbalance between nitric oxide (NO) and reactive oxygen species production in the vascular endothelium are important early steps in atherogenesis, a major socioeconomic health problem. Statins can modify endothelial function and affect atherogenesis by regulating the redox state in the vascular endothelium. [Margaritis 2014]. EA data may be easily accessible indicators of redox properties.

The pleiotropic actions of hydroxymethylglutaryl CoA reductase inhibitors (statins) include anti-inflammatory and antioxidant actions. Statins promote potent systemic antioxidant effects through suppression of distinct oxidation pathways. The major pathways inhibited include formation of myeloperoxidase-derived and nitric oxide-derived oxidants, species implicated in atherogenesis. [Shishehbor 2003] The EA of the statins would be a useful biophysical indicator of the anti-inflammatory and antioxidant of statins, as they are a direct measure of the redox capacity of the statins.

3.3 Active and passive transport models for statins:

A passive diffusion permeation model for atorvastatin, fluvastatin, cerivastatin, pravastatin, pitastatin, lovastatin, rosuvastatin statins based on passive permeation diffusion data for the Corning Transportocells control cells [Li 2014] has been developed. This model is based on the similar model developed for the drug permeability of the blood brain barrier, and incorporates four independent variables, the free energy of water desolvation of the drug, drug lipophilicity based on the free energy of solvation in n-octane, the dipole moment in water, and molecular volume in water. [Fong 2015] The best fit of the passive diffusion rate was for the lactone form, where equation 1 shows a positive correlation with the desolvation free energy in water, dipole moment and molecular volume, and a negative correlation with the lipophilicity. The magnitude of the effects (the coefficients of the independent variables) are highly significant for all variables other than the molecular volume where the standard error is larger than the coefficient. Equations 1-3 can better represented as depending only on desolvation, lipophilicity and dipole, as the molecular volume term is not significantly different from zero. These equations are shown as equation 1a, 2a and 3a. The standard error of the estimate (the dependent variable, the diffusion rate) of 12 compares with the range of rates from 0 to 80 $\mu\text{L}/\text{mg}/\text{min}$. Equation 2 is a much poorer statistical result than equation 1. Based purely on the degree of statistical fit, this data implies that the lactone form is the likely form that permeates the cell membrane by passive diffusion. The corresponding equations for the acid and anionic statins are shown in equations 2, 2a and 3, 3a which have lesser statistical precision than the equations for the lactones. Analysis of the trend of the coefficients does show the relative magnitudes of the independent variables, which can shed mechanistic light on the passive diffusion process.

Comparison of the *trend* of the coefficients for equations 1-3 (or 1a-3a) show that:

- (a) for the statin lactones, it is the dipole moment (the electrostatic interaction between the statin and the protein transport channel that is involved in passive facilitated diffusion, or for the less likely simple diffusion, the interaction between the statin and the cell membrane) that mainly determines the passive diffusion rate
- (b) for the statin acids, it is the lipophilicity (the hydrophobic interaction) and to a lesser extent, the dipole that determines the passive diffusion rate
- (c) for the anionic statins, it is the desolvation energy and the dipole moment that determine the passive diffusion rate. This finding is consistent with the likelihood that the anionic form is unlikely to permeate the cell membrane based on the magnitude of the desolvation energies required for this form (see discussion in section 3.1). The larger contribution from the electrostatic or dipole interaction is consistent with the greater negative charge on the anions, compared with the lactones or acids.
- (d) the zero dependence of equations 1a, 2a, 3a on molecular size of the statins indicates that the mechanism of statins passively diffusing through the cell membrane is via a *facilitated diffusion mechanism* where the statins are passively transported down a trans-membrane protein channel where the size of the channel can accommodate the molecular volumes of all statins which vary from ca. 280 to 440 cm^3/mol . If the passive diffusion process was a purely simple diffusion through the cell membrane, then a correlation would be found with molecular size (as well as desolvation, lipophilicity and dipole moment) as is found with smaller drugs. [Fong 2015]

An analysis of the published K_m rat data from the same experimental study is available for rosuvastatin 7.5, pitastatin 6.3, pravastatin 30.0, fluvastatin 37.6, atorvastatin 4.0 and cerivastatin 7.0 μM . [Shitara 2013] This data has been analysed in equations 4a and 5a.

Equation 4a (lactone form) is more statistically robust than equation 5a (acid form) which might suggest that it is the lactone form that is preferentially taken up by the active OATP transporter, and it is the lipophilicity and dipole moment that governs the thermodynamic binding affinity between the statin lactone and the OATP transporter. This observation is consistent with an essentially electrostatic interaction plus a hydrophobic interaction between the polar and non-polar portions of the statin and the transporter protein respectively. The correlations with $\Delta G_{\text{desolvation}}$ and molecular volume can be omitted since the standard errors are larger than the coefficients which are close to zero, and equation 4a can be better represented by omitting these two variables, giving equation 4b. The K_m correlation with the lipophilicity and dipole moment is consistent with a substrate-protein equilibrium where the substantially desolvated statin substrate lies within, and interacts with the protein environment, and outside the bulk blood plasma environment. If equation 4b is valid then a substantial desolvation must occur before the statin can enter the protein environment and bind to it, and that the hydrophobic interaction is counterbalanced by the electrostatic interaction in the total binding interaction. Equation 5 is of lower precision than equations 4a or 4b, and can be better represented as equation 5b by omitting the molecular volume variable.

- Comparison of the trend of the coefficients for equations 4a and 5b (or 4a and 4b) show:
- for the active transport of the lactones, the dipole or electrostatic interaction and to a lesser extent the lipophilicity or hydrophobic effect determines the binding interaction between the statin lactones and the OATP transporter
 - for the active transport of the acids, the dipole or electrostatic interaction and the lipophilicity or hydrophobic effect determine the binding interaction, with a smaller contribution from the desolvation energy.

However these *indicative* equations (which are not strongly robust in view of the limited number of experimental data points) can only be supportive evidence, but data from other literature studies (as discussed in sections 2.1, 2.4, 2.6) does support the hypothesis that the lactone form is the likely form that is responsible for the majority of the passive diffusion and active transport by OATP transporters. It is possible that both neutral acid and lactone forms are competitively transported across the cell membrane. In view of the molecular volume or size of these statins, it is likely that the transport mechanism is a facilitated passive diffusion process. From equation 4b, it can be estimated that the average polar (electrostatic) interaction between the statins and the OATP transporter protein is *ca. 4 times as strong* as the hydrophobic binding interaction.

For the statin lactones: Equation 1

$$\text{Passive diffusion rate} = 0.5 \Delta G_{\text{desolvation}} - 1.6 \Delta G_{\text{lipophilicity}} + 8.9 \text{ Dipole Moment} + 0.5 \text{ Molecular Volume} + 43.9$$

Where $R^2 = 0.954$, $SEE = 11.9$, $SE(\Delta G_{\text{desolvation}}) = 0.15$, $SE(\Delta G_{\text{lipophilicity}}) = 1.05$, $SE(\text{Dipole Moment}) = 2.99$, $SE(\text{Molecular Volume}) = 2.16$

For the statin lactones: Equation 1a

$$\text{Passive diffusion rate} = 0.5 \Delta G_{\text{desolvation}} - 1.5 \Delta G_{\text{lipophilicity}} + 9.2 \text{ Dipole Moment} + 43.1$$

Where $R^2 = 0.953$, $SEE = 9.8$, $SE(\Delta G_{\text{desolvation}}) = 0.09$, $SE(\Delta G_{\text{lipophilicity}}) = 0.76$, $SE(\text{Dipole Moment}) = 2.10$, $SE(\text{Molecular Volume}) = 2.16$

For the statin acids: Equation 2

$$\text{Passive diffusion rate} = 0.5 \Delta G_{\text{desolvation}} + 11.4 \Delta G_{\text{lipophilicity}} + 3.8 \text{ Dipole Moment} + 0.4 \text{ Molecular Volume} + 49.8$$

Where $R^2 = 0.772$, $SEE = 26.6$, $SE(\Delta G_{\text{desolvation}}) = 3.19$, $SE(\Delta G_{\text{lipophilicity}}) = 5.24$, $SE(\text{Dipole Moment}) = 8.26$, $SE(\text{Molecular Volume}) = 0.20$

For the statin acids: Equation 2a

$$\text{Passive diffusion rate} = 0.3 \Delta G_{\text{desolvation}} + 8.0 \Delta G_{\text{lipophilicity}} + 4.8 \text{ Dipole Moment} + 122.6$$

Where $R^2 = 0.334$, $SEE = 37.1.6$, $SE(\Delta G_{\text{desolvation}}) = 4.45$, $SE(\Delta G_{\text{lipophilicity}}) = 6.92$, $SE(\text{Dipole Moment}) = 11.5$,

For the statin anions: Equation 3

$$\text{Passive diffusion rate} = 1.2 \Delta G_{\text{desolvation}} + 0.9 \Delta G_{\text{lipophilicity}} + 2.2 \text{ Dipole Moment} - 0.2 \text{ Molecular Volume} - 72.0$$

Where $R^2 = 0.688$, $SEE = 31.1$, $SE(\Delta G_{\text{desolvation}}) = 2.21$, $SE(\Delta G_{\text{lipophilicity}}) = 1.32$, $SE(\text{Dipole Moment}) = 4.43$, $SE(\text{Molecular Volume}) = 0.35$

For the statin anions: Equation 3a

$$\text{Passive diffusion rate} = 2.1 \Delta G_{\text{desolvation}} + 0.7 \Delta G_{\text{lipophilicity}} + 1.3 \text{ Dipole Moment} - 174.0$$

Where $R^2 = 0.646$, $SEE = 27.0$, $SE(\Delta G_{\text{desolvation}}) = 1.24$, $SE(\Delta G_{\text{lipophilicity}}) = 1.07$, $SE(\text{Dipole Moment}) = 3.53$

For the active transport of statin lactones by OATP transporter: Equation 4a

$$K_m = -0.3 \Delta G_{\text{desolvation}} + 2.3 \Delta G_{\text{lipophilicity}} - 3.8 \text{ Dipole Moment} - 0.0 \text{ Molecular Volume} + 96.3$$

Where $R^2 = 0.972$, $SEE = 5.40$, $SE(\Delta G_{\text{desolvation}}) = 0.39$, $SE(\Delta G_{\text{lipophilicity}}) = 1.34$, $SE(\text{Dipole Moment}) = 0.89$, $SE(\text{Molecular Volume}) = 0.15$

For the active transport of statin lactones by OATP transporter: Equation 4b

$$K_m = + 2.7 \Delta G_{\text{lipophilicity}} - 4.0 \text{ Dipole Moment} + 88.5$$

Where $R^2 = 0.952$, $SEE = 4.09$, $SE(\Delta G_{\text{lipophilicity}}) = 0.64$, $SE(\text{Dipole Moment}) = 0.58$

For the active transport of statin acids by OATP transporter: Equation 5

$$K_m = -1.4 \Delta G_{\text{desolvation}} + 4.0 \Delta G_{\text{lipophilicity}} + 4.3 \text{ Dipole Moment} - 0.1 \text{ Molecular Volume}$$

Where $R^2 = 0.811$, $SEE = 14.1$, $SE(\Delta G_{\text{desolvation}}) = 1.80$, $SE(\Delta G_{\text{lipophilicity}}) = 3.13$, $SE(\text{Dipole Moment}) = 5.65$, $SE(\text{Molecular Volume}) = 0.11$

For the active transport of statin acids by OATP transporter: Equation 5a

$$K_m = -1.3 \Delta G_{\text{desolvation}} + 5.1 \Delta G_{\text{lipophilicity}} + 3.1 \text{ Dipole Moment}$$

Where $R^2 = 0.674$, $SEE = 13.0$, $SE(\Delta G_{\text{desolvation}}) = 1.65$, $SE(\Delta G_{\text{lipophilicity}}) = 2.65$, $SE(\text{Dipole Moment}) = 5.10$,

Gemfibrozil and cyclosporin are known to be inhibitors of the OATP1B1 transporter, but not substrates, and to increase statin plasma concentrations. [Kalliokoski 2009, Goosen 2007, Whitfield 2011, Koenen 2011] The area under the curve fold ratio (AUCR) for gemfibrozil and cyclosporin are known [Kalliokoski 2009, table 4] and are analysed in equations 6a, 6b and 7a, 7b for the inhibition of eight statin acid and lactones by gemfibrozil and cyclosporin respectively. It is noted that there is some smaller contributions from CYP enzyme inhibition other than OATP1B1 alone, for example for atorvastatin, cerivastatin and simvastatin where some CYP3A4 inhibition is thought to be involved for cyclosporin inhibition, and where some CYP2C8 inhibition is thought to be involved with gemfibrozil inhibition of OATP1B1. Hence the AUCR is not a sensitive or precise indicator of the inhibition of statin-transporter processes, but may shed light on systemic factors. These equations commonly show zero dependence on molecular volume, and equations 6b and 7b (statin lactones) also show zero dependence on $\Delta G_{\text{desolvation}}$ (shown as struck out). The correlations with the statin lactones are statistically stronger than for the acids, similar to the previous transporter correlation

equations 4a-5a, implying a preference for lactone transport over the acid form of statins. There are ample literature references supporting a major role for lactones being involved in OATP transport along with the acid form. [Kalliokoski 2009, Goosen 2007, Whitfield 2011, Koenen 2011, Schneck 2004, Jacobson 2000] The AUCR is inversely dependent on the clearance, and dependent on the dose administered and its bioavailability (see Table 1). For orally administered drugs, the bioavailability is dependent on many complex factors, so transporter inhibition effects can be confounded, which may explain the relatively small dependencies on lipophilicity and dipole moment. For example cyclosporin is an inhibitor of influx and efflux transporters such as OATP1B1, OATP1B3, MDR1, MDRP2, as well as CYP3A4. The AUCR values vary within the range of 1.5-4.4 and 3.5-20.0 for gemfibrozil and cyclosporin inhibition respectively. Mechanistically these equations are consistent with a facilitated transport channel for the statins which can be inhibited with gemfibrozil and cephalosporin, where the protein transporter channel size easily accommodates all the statins, the statins are essentially desolvated before entering the channel, and interaction between the protein and statins is predominantly a modest lipophilic (hydrophobic) interaction, coupled with a modest dipolar electrostatic interaction. These equations confirm the notion that OATP1B1 transport is a factor in statin hepatic uptake and systemic bioavailability.

For the OATP1B1 transporter inhibition by *gemfibrozil* of statin *acids*: Equation 6a

$$\text{AUCR} = 0.2 \Delta G_{\text{desolvation}} + 0.3 \Delta G_{\text{lipophilicity}} - 0.1 \text{ Dipole Moment} + 0.0 \text{ Molecular Volume} - 1.3$$

Where $R^2 = 0.827$, $SEE = 0.70$, $SE(\Delta G_{\text{desolvation}}) = 0.08$, $SE(\Delta G_{\text{lipophilicity}}) = 0.13$, $SE(\text{Dipole Moment}) = 0.21$, $SE(\text{Molecular Volume}) = 0.0$

For the OATP1B1 transporter inhibition by *gemfibrozil* of statin *lactones*: Equation 6b

$$\text{AUCR} = -0.0 \Delta G_{\text{desolvation}} + 0.4 \Delta G_{\text{lipophilicity}} + 0.2 \text{ Dipole Moment} + 0.0 \text{ Molecular Volume} - 2.6$$

Where $R^2 = 0.882$, $SEE = 0.58$, $SE(\Delta G_{\text{desolvation}}) = 0.40$, $SE(\Delta G_{\text{lipophilicity}}) = 0.10$, $SE(\text{Dipole Moment}) = 0.08$, $SE(\text{Molecular Volume}) = 0.01$

For the OATP1B1 transporter inhibition by *cyclosporin* of statin *acids*: Equation 7a

$$\text{AUCR} = 0.6 \Delta G_{\text{desolvation}} - 0.2 \Delta G_{\text{lipophilicity}} - 2.2 \text{ Dipole Moment} + 0.0 \text{ Molecular Volume} - 9.6$$

Where $R^2 = 0.404$, $SEE = 6.23$, $SE(\Delta G_{\text{desolvation}}) = 0.74$, $SE(\Delta G_{\text{lipophilicity}}) = 1.16$, $SE(\text{Dipole Moment}) = 1.84$, $SE(\text{Molecular Volume}) = 0.04$

For the OATP1B1 transporter inhibition by *cyclosporin* of statin *lactones*: Equation 7b

$$\text{AUCR} = 0.0 \Delta G_{\text{desolvation}} + 0.2 \Delta G_{\text{lipophilicity}} - 0.1 \text{ Dipole Moment} + 0.0 \text{ Molecular Volume} + 2.4$$

Where $R^2 = 0.988$, $SEE = 0.88$, $SE(\Delta G_{\text{desolvation}}) = 0.05$, $SE(\Delta G_{\text{lipophilicity}}) = 0.02$, $SE(\text{Dipole Moment}) = 0.08$, $SE(\text{Molecular Volume}) = 0.00$

3.4 Models for statins as inhibitors of CYP enzymes:

CYP2C8 is one of the major drug-metabolizing CYP enzymes. It is important in the metabolism of many drugs including paclitaxel, rosiglitazone, pioglitazone, repaglinide and amodiaquine. In humans, gemfibrozil has markedly increased the plasma concentrations of the CYP2C8 substrate cerivastatin 5-6 times, and also increases the plasma levels of simvastatin acid and lovastatin acid but with little effect on the lactone forms. The *in vitro* inhibition (K_i) of simvastatin 7.1, simvastatin acid 41.1, lovastatin 8.4, lovastatin acid 48.9, fluvastatin 18.9, pravastatin >50, cerivastatin 31.7, rosuvastatin >50 and atorvastatin 15.9 μM

on the CYP2C8 model reaction, paclitaxel 6 α -hydroxylation, in human liver microsomes has been studied. [Tornio 2005] The outer membrane of mitochondria have integral proteins called porins that allow molecules of up to 5000 Da to freely diffuse through, so the concentration of small molecules in the intermembrane space is the same as in the cytosol. The inner membrane contains proteins including those responsible for redox reactions of oxidative phosphorylation. A model equation to relate the K_i values to the free energy of desolvation, the dipole moment and the electron affinity (EA) is shown below as equation 8. The EA is a measure of the ease of reduction of a substrate, as occurs in the oxidation-reduction process occurring in cytochrome enzymes, eventually forming in this case, 6 α -hydroxypaclitaxel. Molecular volume is an irrelevant factor in this case as the statins are well under the 5000 Da limit. Equation 8 shows a strong relationship for the acids and lactones, particularly for the EA.

Simvastatin acid and lovastatin acid were ca. six times weaker inhibitors of CYP2C8 than their lactone counterparts, which is consistent with the higher EA for acids compared to lactones (see Table 2) which indicate that the acids are metabolised (hydroxylated) more readily than the lactone forms, which then act as stronger inhibitors when bound to CYP2C8. In terms of likely side effects caused by statin inhibition of CYP2C8, the K_i values are about 10-100 times the peak plasma concentrations of these statins at steady state and standard daily doses. [Tornio 2005]

For the statin inhibition of paclitaxel oxidation by CYP2C8 (Tornio): Equation 8

$$K_i = 3.7 \Delta G_{\text{desolvation}} + 5.7 \Delta G_{\text{lipophilicity}} - 6.7 \text{ Dipole Moment} + 30.4 \text{ Electron Affinity} + 38.2$$

Where $R^2 = 0.964$, $SEE = 5.4$, $SE(\Delta G_{\text{desolvation}}) = 0.64$, $SE(\Delta G_{\text{lipophilicity}}) = 1.36$, $SE(\text{Dipole Moment}) = 1.66$, $SE(\text{Electron Affinity}) = 7.24$

The inhibitory effects of eight statin acids and lactones on CYP2C8, 2C9/10 and 2C19 and 3A4/5 metabolism and MDR1 transport activities with human liver microsomes and MDR1 over-expressing cell lines have been reported. Overall the acid forms showed little or no inhibitory effects, and the lactones showed small effects with the exception of CP3A4/5 activity, with IC_{50} values of atorvastatin 5.6, cerivastatin 8.1, fluvastatin 14.9, simvastatin 15.2, rosuvastatin 20.7 and lovastatin 24.1 μM . MDR1 mediated transport of digoxin was inhibited only by the lactones, and the order was correlated with the same order for the inhibition of CYP3A4/5. [Sakaeda 2006] The paclitaxel 6 α -hydroxylation CYP2C8 IC_{50} inhibition data is similar to the results obtained by Tornio, but Tornio has K_i data, intrinsic constants which are more accurate than IC_{50} data (extrinsic constants more dependent on experimental variables). The Sakaeda data analysis is shown in equation 9, which is far less significant than equation 8, but basically shows similar dependencies on the four dependent variables. *Of course these two data sets come from different experimental studies, but the agreement gives added confidence that the observed relationships are valid.* The substantial difference between equations 9 and 10 is the lesser inhibitory dependency on the lipophilicity and electron affinity of the statin lactones in equation 10. Equation 11 shows the IC_{50} relationship for the 3-hydroxypaclitaxel CYP3A4/5 inhibition, which gives a similar result to equations 9 and 10, but is a marginally more statistically significant result. Equation 11 shows a twice the dependency on EA for the CYP3A4 compared to the dependency on EA for the CYP2C8, which might suggest the former enzyme system may be a more potent metabolizer of statin lactones.

For the statin *acids* inhibition of paclitaxel oxidation by CYP2C8 (Sakaeda): Equation 9

$$\text{IC}_{50} = 6.6 \Delta G_{\text{desolvation}} + 28.6 \Delta G_{\text{lipophilicity}} - 5.2 \text{ Dipole Moment} + 210.5 \text{ Electron Affinity} + 122.7$$

Where $R^2 = 0.921$, $\text{SEE} = 11.0$, $\text{SE}(\Delta G_{\text{desolvation}}) = 6.86$, $\text{SE}(\Delta G_{\text{lipophilicity}}) = 18.46$, $\text{SE}(\text{Dipole Moment}) = 5.58$, $\text{SE}(\text{Electron Affinity}) = 152.8$

For the statin *lactones* inhibition of paclitaxel oxidation by **CYP2C8** (Sakaeda): Equation 10

$$\text{IC}_{50} = 6.9 \Delta G_{\text{desolvation}} + 10.9 \Delta G_{\text{lipophilicity}} - 10.0 \text{ Dipole Moment} + 51.6 \text{ Electron Affinity} - 110.8$$

Where $R^2 = 0.871$, $\text{SEE} = 15.0$, $\text{SE}(\Delta G_{\text{desolvation}}) = 2.9$, $\text{SE}(\Delta G_{\text{lipophilicity}}) = 3.1$, $\text{SE}(\text{Dipole Moment}) = 6.4$, $\text{SE}(\text{Electron Affinity}) = 37.2$

For the statin *lactones* inhibition of paclitaxel oxidation by **CYP3A4/5** (Sakaeda): Equation 11

$$\text{IC}_{50} = 11.0 \Delta G_{\text{desolvation}} + 11.2 \Delta G_{\text{lipophilicity}} - 16.2 \text{ Dipole Moment} + 111.9 \text{ Electron Affinity} - 20.8$$

Where $R^2 = 0.775$, $\text{SEE} = 23.4$, $\text{SE}(\Delta G_{\text{desolvation}}) = 4.54$, $\text{SE}(\Delta G_{\text{lipophilicity}}) = 4.83$, $\text{SE}(\text{Dipole Moment}) = 9.9$, $\text{SE}(\text{Electron Affinity}) = 57.89$

The electrochemical peak reduction potentials E_p for the statin acids are related to the electron affinities EA. There are many examples of linear relationships between E_p and EA for reversible reactions. However the relationship does not strictly apply for irreversible reactions, which is the situation for the electrochemical studies of the statins (except pravastatin which can be reversible or irreversible depending on the conditions). Equation 9 describes the statin acids inhibition of paclitaxel oxidation by CYP2C8, which is a competitive inhibition of the complex redox processes involved in CYP2C8 metabolism. Since E_p describes the ease of redox reduction of the statin acids under electrochemical conditions, it may be instructive to see if there is any relationship between IC_{50} values for paclitaxel oxidation by CYP2C8 and the known E_p values under conditions where the acid form is dominant, at pH 5 (equation 12). It can be seen that equations 9 and 12 are very similar, except that the significant dipole moment dependencies are reversed. If this observation is physically valid, and not a statistical aberration, this may be a result of the electrodic electrochemical environment being quite different from a purely aqueous environment for the EA calculations. The dependency on desolvation energy is not statistically significant, and can be omitted with an improvement in precision. *Equations 9 and 12 do support the importance of electron transfer processes being important for CYP metabolism of statins*, and that the more easily obtained EA values can be used as substitutes for experimentally derived reduction potentials during drug screening evaluations.

For the statin *acids* inhibition of paclitaxel oxidation by **CYP2C8** (Sakaeda): Equation 12

$$\text{IC}_{50} = -2.6 \Delta G_{\text{desolvation}} + 6.4 \Delta G_{\text{lipophilicity}} + 19.0 \text{ Dipole Moment} + 86.0 \text{ Peak Reduction Potential} + 13.8$$

Where $R^2 = 0.916$, $\text{SEE} = 11.9$, $\text{SE}(\Delta G_{\text{desolvation}}) = 3.5$, $\text{SE}(\Delta G_{\text{lipophilicity}}) = 2.4$, $\text{SE}(\text{Dipole Moment}) = 7.9$, $\text{SE}(\text{Peak Reduction Potential}) = 44.6$

Conclusions

The neutral lactone and acid statin species are preferentially transported across the cell membrane and consequentially preferentially metabolised and cleared. The preferred cellular uptake of statin lactones has implications for cytotoxicity in muscle tissue and other side effects. The uptake mechanism is a combination of passive facilitated diffusion and active

permeation by OATP transporters. Equations 1-5 describe how passive diffusion facilitated uptakes rates, and binding affinity between statins and OATP transporters are related to the desolvation, lipophilicity, dipole moment and molecular volume components of a previously described membrane permeation model, [Fong 2015] Equations 6-7 describe similar relationships between the cyclosporin and gemfibrozil competitive inhibition of statin-OATP1B1. Equations 8-12 describe the relationship between IC_{50} and the desolvation, lipophilicity, dipole moment and either the electron affinity, EA, or the electrochemical peak reduction potential for the statin inhibition of paclitaxel oxidation by CYP2C8. These equations suggest that electron affinity, a measure of reduction potential, is a useful indicator of potential drug-drug interactions (DDI). An examination of drugs known to cause DDI with statins show reduction potential and cellular uptake properties are useful predictors of DDI. These statistical models, supported by literature evidence, indicate the statin lactones play a previously unrecognized major role in statin therapeutics, and in side effects, cytotoxicity and DDI.

Material and methods

All calculations were carried out as previously described [Fong 2014] using the Gaussian 09 package at the B3LYP/6-31G*(6d, 7f) level of theory with optimised geometries, as this level has been shown to give accurate electrostatic atomic charges, and was used to optimize the IEFPCM/SMD solvent model. With the 6-31G* basis set, the SMD model achieves mean unsigned errors of 0.6 - 1.0 kcal/mol in the solvation free energies of tested neutrals and mean unsigned errors of 4 kcal/mol on average for ions. [Marenich 2009] The 6-31G* basis set has been used to calculate absolute free energies of solvation and compare these data with experimental results for more than 500 neutral and charged compounds. The calculated values were in good agreement with experimental results across a wide range of compounds. [Rayne 2010, Rizzo 2006]

Adding diffuse functions to the 6-31G* basis set (ie 6-31⁺*) had no significant effect on the solvation energies with a difference of ca 1% observed in solvents for the fluvastatin anion, which is within the literature error range for the IEFPCM/SMD solvent model. This is consistent with the finding [Treitel 2004] diffuse functions had a negligible effect on energy, geometry and charges for anions where conjugation or delocalisation of the negative charge was occurring.

It is noted that high computational accuracy for each species in different environments is not the focus of this study, but comparative differences between various species is the aim of the study. The use of various literature values for passive diffusion rates, K_m , IC_{50} , AUCR etc to develop the multiple regression equations have much higher uncertainties than the calculated molecular properties.

Table 1. Biophysical, metabolic and transport properties of statins

	Bio-avail. %	Half Life Hrs	Vol. of Distributn L	Log D	pK _a Acid	Active Metabolites	CYP Substrate	CYP 3A4/5 Binding ^C IC ₅₀ μM	OATP Transport*	OATP Transport K _m μM MM	Binding Energy ^A HMG-CoA Reduct. kcal/mol {IC ₅₀ ^A nM}	Plasma Protein Binding %
Atorvastatin	12	14	381	1.0-1.25	4.6	yes	3A4	5.6 Lactone	1B1*	0.6 ^F , 0.77*, 0.3 ^I	-10.9 {1.7}	98

								74.6 Acid	1B3* 2B1* 2B1 ^E	0.73*, 2.0 ^I 2.84* 0.2 ^E (heart)		
Fluvastatin	24 (9-50)	2.3	30	1.0- 1.25	5.5	no	2C9	14.9 Lactone Nil Acid	1B1 1B3 2B2	1.4-3.5 ^G , 7.0 ^G 0.7 ^G (37.6 ^D OATP2)	-9.0 {0.3}	98
Lovastatin	5	3	na	3.91* 1.51#	4.3	yes	3A4	24.1 Lactone Nil Acid	1B1		{0.6}	95
Pitastatin	60	12	148	1.50	4.7	Minimal Lactone	2C8 2C9 Glu	67.2 Lactone 3A4/5 Nil Acid	1A2 1B1 1B3 2B1	3.0 ^H 3-4 ^H 3-4 ^H 1.2 ^H (3.9 ^B OATP2)		96
Pravastatin	18	1.3- 2.7	35	-0.47	4.7	minimal	3A4 Sulf	73.7 Lactone Nil Acid	1B1 2B1	14-34 ^H 2 ^H (OATP2) 32.3 ^D	-9.7 {2.3}	50
Rosuvastatin	20	19	134	-0.25 to - 0.5	4.6	yes minimal	2C9 Excr	20.7 Lactone Nil Acid	1A2 1B1 1B3, 2B1	3.0 ^H 9 ^H 10 ^H 2 ^H 7.3 ^B (OATP2)	-12.3	90
Simvastatin	<5	3	na	4.40* 1.80#	4.2	yes	3A4	15.2 Lactone 12.0 Acid	1B1		{0.12}	95
Cerivastatin	60	2.5	21	1.5- 1.75	3.9	yes	3A4, 2C8	8.1 Lactone Nil Acid	1B1	4 ^H 4.3 ^B (OATP2)	-11.4	99

Footnotes:

Data from McFarland A, et al, Molecular Mechanisms Underlying the Effects of Statins in the Central Nervous System, Int.J.Molec.Sci., 2014,15,20607 and Schacter M, Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update, Fundamental & Clinical Pharmacology 19 (2004) 117–125 and White MC, A review of the pharmacologic and pharmacokinetic aspects of rosuvastatin, J. Clin. Pharmacol., 2002, 42, 963.

* Karlgren M, et al, Classification of Inhibitors of Hepatic Organic Anion Transporting Polypeptides (OATPs): Influence of Protein Expression on Drug–Drug Interactions, J. Med. Chem, 2007, 55, 4740. Km (μM) derived from Michaelis Menten (MM) calculations
^A Binding energy (isothermal calorimetry) and IC₅₀ refer to binding of statins with HMG-CoA Reductase: Freire E, Do enthalpy and entropy distinguish first in class from best in class?, Drug Discovery Today, 2008, 13, 869.

Swinney DC, Biochemical mechanisms of drug action: what does it take for success? Nature Reviews, Drug Discovery, 2004, 3, 801.

^B Fujino H, Kojima J, Ch 5, p 109, Focus on Statin Research: Drug metabolism and transporter properties of statins, in Focus on Statin Research, Wong BA, ed, Nova Science Publisher, 2006, NY.

^C Sakaeda T, et al, Effects of acid and lactone forms of eight HMGCoA-reductase inhibitors on CYP-mediated metabolism and MDRI-mediated transport, Pharm, Res., 2006, 23, 506. Statin inhibitory effects were tested on paclitaxel 3-hydroxylation by CYP3A4/5.

^D Ohtawa M, et al, Cellular uptake of fluvastatin, an inhibitor of HMGCoA reductase, by rat cultured hepatocytes and human aortic Br. J. Clin. Pharmacol., 1999, 47, 383.

^E Grube M, et al, Organic anion transporting polypeptide 2B1 is a high-affinity transporter for atorvastatin and is expressed in the human heart, Clin. Pharmacol. Ther. 2006, 80, 607.

^F Sharma P, Butters CJ, Smith V, Elsby R, Surry D, Prediction of the in vivo OATP1B1-mediated drug-drug interaction potential of an investigational drug against a range of statins, Eur. J. Pharm. Sci., 2012, 47, 244.

^G Kalliokoski A, Niemi M, Impact of OATP transporters on pharmacokinetics, Br. J. Pharmacol. 2009, 158, 693.

^H Roth M, Obaidat A, Hagenbuch B, OATPs, OATs and OCTs: the organic anion and cation transporters of the (human) *SLCO* and *SLC22A* gene superfamilies, Br. J. Pharmacol. 2012, 165, 1650.

^I Mieziewski B, et al, In vitro Systems to Assess the Potency of Selected Uptake Transporter Inhibitors, 2012, AAPS/M1321, HEK293 cells.

CYP Substrate: Glu = mostly glucuronidation, Sulf = mostly sulfuration, Excr = mostly excreted unchanged

* = lactone, # = acid.

Table 2. Solvation energies, lipophilicities, dipole moments, molecular volumes, ionization energies, electron affinities, reduction potentials of statins and interacting drugs

Statin	Solvation Energy Water - kcal/mol	Lipophilicity kcal/mol	Dipole Water D	Molecular Volume cm ³ /mol	IE eV Water	EA eV Water (Reduction Potential Volts pH 5) {Absolute Reduction Potl Calc}
Rosuvastatin Anion 90°	74.2	-37.3	38.6	331	5.8	1.7

Fluvastatin Anion 90°	85.1	-37.7	41.4	283	5.3	1.4
Atorvastatin 90° Anion	75.7	-40.7	35.0	374	4.6	1.0
Cerivastatin 90° Anion	94.1	-39.0	46.6	278	5.5	1.0
Pitavastatin 90° Anion	93.8	-40.5	45.7	299	5.5	1.8
Lovastatin Anion	87.8	-34.8	21.7	335	5.4	1.0
Pravastatin Anion	104.3	-40.8	37.5	356	5.4	0.8
Simvastatin Anion	84.5	-33.2	21.2	322	5.4	1.0
Rosuvastatin Acid 90°	32.7	-20.9	10.1	301	5.9	1.8 (1.18) {1.37}
Fluvastatin Acid 90°	23.7	-16.0	8.6	308	5.3	1.4 (0.64) {1.44}
Atorvastatin Acid 90°	26.7	-21.6	8.5	439	5.0	1.9 (0.92) {1.58}
Cerivastatin 90° Acid	37.2	-17.8	11.5	425	5.6	1.1 {1.32}
Pitavastatin Acid 90°	26.4	-18.1	6.2	335	4.8	1.5 (1.21) {1.52}
Lovastatin Acid	25.5	-15.0	5.1	340	5.4	1.1 (1.49) {1.63}
Pravastatin Acid	35.3	-17.0	9.4	298	5.4	0.8 (1.32) {1.38}
Simvastatin Acid	25.4	-14.6	5.1	378	5.4	1.0 (1.12) {1.70}
Rosuvastatin Lactone 90°	25.7	-16.8	7.7	316	6.2	4.6* {2.01}
Fluvastatin Lactone 90°	14.2	-16.4	1.5	304	5.0	0.7 {1.42}
Atorvastatin Lactone 90°	26.7	-23.3	5.5	355	5.0	0.8 {1.42}
Cerivastatin Lactone 90°	16.4	-15.9	10.1	342	5.8	1.7 {1.55}
Pitavastatin Lactone 90°	19.0	-17.5	9.1	300	5.6	1.9 {1.56}
Lovastatin Lactone	18.1	-14.5	4.0	292	5.4	1.0 {1.59}
Pravastatin Lactone	24.2	-15.6	4.8	295	5.4	0.6 {1.66}
Simvastatin Lactone	20.7	-16.3	4.2	349	5.1	0.5 {1.69}
Gemfibrozil	12.2	-10.4	3.9	198	6.0	0.6
Gemfibrozil -Ion	74.1	-32.2	27.6	200	5.4	0.3
Fenofibrate	10.5	-14.0	8.2	239	6.1	2.6
Fenofibric Acid	18.0	-13.4	7.5	269	6.7	2.4
Ezetimibe	16.1	-16.1	6.4	294	5.5	0.7
Warfarin	15.8	-13.7	3.6	185	5.9	1.7
Warfarin -Ion	58.2	-29.1	5.4	267	5.1	1.1
Verpamil	9.5	-12.4	8.2	416	5.2	0.6
Verpamil +Ion	62.4	-37.1	9.9	343	5.6	0.7
Niacin	12.9	-6.1	4.3	78	7.0	2.1
Niacin -Ion	70.5	-28.4	12.4	89	5.9	1.1
Mibefradil	20.0	-18.7	8.2	381	5.5	0.6
Mibefradil +Ion	72.7	-43.2	14.8	385	5.9	0.7
Midazolam	13.2	-12.1	4.2	246	5.9	2.0
Cyclosporin	54.0	-35.3	1.5	889	5.5	1.3
Digoxin	43.0	-23.1	18.3	515	1.4	1.4
Cimetidine	20.2	-15.9	6.9	205	5.9	2.0
Colchicine	15.7	-11.2	6.0	268	5.4	2.1
Ubiquinone	5.2	-26.3	3.5	575	5.7	3.5

Footnotes to Table 2:

Solvation energies are calculated using the SMD - Polarizable Continuum Model (IEFPCM), Unified Force Field, scaled van der Waals surface cavity. Solvation (free) energies are the differences between the energies of the optimised statin in the gas phase and in the particular solvent. A. V. Marenich, C. J. Cramer, and D. G. Truhlar, J. Phys. Chem. B, 2009, 113, 6378-96.

Lipophilicity is the solvation free energies in n-octane, essentially a repulsive energy.

IE, EA: Vertical ionization energy and electron affinity in eV. Calculated from by the SCF difference method for anionic form as $IE = E(M^-) - E(M)$ and $EA = E(M^-) - E(M^{\cdot-})$ at the optimised geometry of M^- , or for the neutral acid form as $IE = E(M) - E(M^+)$ and

$EA = E(M) - E(M^{\cdot})$ at the optimised geometry of M .

The IE and EA for rosuvastatin lactone are clearly outliers from the range of values for other statins, as well as the acid and anion forms, and appears to be related to the lower atomic charges on the methanesulphonate group, which is unique to the approved statins.

[Reduction potential] are literature electrochemical values at ~pH 5. See text section 3.2.

{Absolute reduction potential} are calculated values as per Topol IA, et al, Experimental determination and calculations of redox potential descriptors of compounds directed against retroviral zinc fingers: Implications for rational drug design, Protein Science, 2001, 10, 1434.

Values in cm^3/mol are molecular volumes in water defined as the volume inside a contour of 0.001 electrons/Bohr³ density.

The angles 90° etc refer to the conformational angles between the 4-FC₆H₅- group and the relevant heterocyclic ring where applicable.

Table 3. Metabolic clearance rates for statins and major metabolic enzymes involved

	Metabolic Clearance Acid	Metabolic Clearance Lactone	Clearance Ratio Lact/Acid	CYP Enzymes Acid	CYP Enzymes Lactone
Rosuvastatin	1	71	71	-	CYP3A4
Fluvastatin	33	226	7	CYP2C9	CYP3A4

Atorvastatin	26	1892	73	CYP3A4	CYP3A4
Cerivastatin	21	622	30	CYP2C8 CYP3A4	CYP3A4
Pitavastatin	3	5	2	CYP2C9	CYP3A4
Simvastatin	28	1959	70	CYP3A4	CYP3A4

Footnotes:

Data from Fujino H, Kojima J, Ch 5, p 109, Focus on Statin Research: Drug metabolism and transporter properties of statins, in Focus on Statin Research, Wong BA, ed, Nova Science Publisher, 2006, NY.
Metabolic clearances, CL_{int} are $\mu\text{L}/\text{min}/\text{mg}$ protein.

References

- Alder NN, Theg SM, Energy use by biological protein transport pathways, Trends in Biochem. Sci., 2003, 28, 442.
- Altnoz S, Uyar B, Electrochemical behaviour and voltammetric determination of rosuvastatin calcium in pharmaceutical preparations using a square-wave voltammetric method, Anal. Methods, 2013, 5, 5709.
- Alupei MC, Licarete E, Cristian FB, Banciu M, Cytotoxicity of lipophilic statins depends on their combined actions on HIF-1 α expression and redox status in B16.F10 melanoma cells, Anti-cancer drugs 01/2014; DOI: 10.1097/CAD.0000000000000065
- Backman JT, Kyrklund C, Neuvonen M, Neuvonen PJ, Gemfibrozil greatly increases plasma concentrations of cerivastatin, Clin. Pharmacol. Ther. 2002, 72, 685.
- Bellostra S, Paoletti R, Corsini A, Safety of statins, Circulation, 2004, 109 (suppl. III-50)
- Boudker O, et al, Coupling substrate and ion binding to extracellular gate of a sodium-dependent aspartate transporter, Nature, 2007, 445, 387.
- Brandis K, Acid base physiology, <http://www.anaesthesiaMCQ.com>
- Catapano AL, Pitavastatin - pharmacological profile from early phase studies, Atheroscler. Suppl. 2010, 11, 3
- Chaplin MF, Bucke C, Enzyme Technology, Cambridge University Press, 1990, Ch 1, p7.
- Chen C, et al, Differential interaction of 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors with ABCB1, ABCC2, and OATP1B1, Drug Metab. Dispos., 2005, 33, 2477.
- Chen C, et al, P-glycoprotein Has Differential Effects on the Disposition of Statin Acid and Lactone Forms in *mdr1a/b* Knockout and Wild-Type Mice, Drug Metab. Dispos., 2007, 35, 1725.
- Coruh O, Ozkan SA, Determination of the antihyperlipidemic simvastatin by various voltammetric techniques in tablets and serum samples, Pharamzie, 2006, 61, 285.
- Dogan-Topal B, Bozal B, Demircigil BT, Uslu B, Ozkan SA, Electroanalytical studies and simultaneous determination of amlodipidine besylate and atorvastatin calcium in binary mixtures using first derivative of radio-voltammetric methods, Electroanalysis, 2009, 21, 2427.
- Dostalek M, Sam WJ, Paryani KR, Macwan JS, Gohh RY, Akhlaghi F, Diabetes mellitus reduces the clearance of atorvastatin lactone: results of a population pharmacokinetic analysis in renal transplant recipients and in vitro studies using human liver microsomes, Clin.Pharmacokinet., 2012, 51, 591
- Eghbal MA, Abdoli M, Azarmi Y, Efficiency of hepatocyte pretreatment with coenzyme Q10 against statin toxicity, Arh. Hig. Rada. Toksikol. 2014, 65, 101.
- Fong CW, Statins in therapy: Understanding their hydrophilicity, lipophilicity, binding to 3-hydroxy-3-methylglutaryl-CoA reductase, ability to cross the blood brain barrier and metabolic stability based on electrostatic molecular orbital studies, Europ. J. Med. Chem., 2014, 85, 661.
- Fong CW, Permeability of the Blood–Brain Barrier: Molecular Mechanism of Transport of Drugs and Physiologically Important Compounds, J. Membrane Biol. Feb., 2015, DOI 10.1007/s00232-015-9778-9

Fricker G, Fahr A, Mechanisms of hepatic transport of cyclosporin A: an explanation for its cholestatic action? *Yale J. Biol. Med.*, 1997, 70, 379.

Fujino H, Saito T, Tsunenari Y, Kojima J, Sakaeda T, Metabolic properties of the acid and lactone forms of HMG-CoA reductase inhibitors, *Xenobiotica*. 2004, 34, 961.

Fujino H, Kojima J, Ch 5, p 109, Focus on Statin Research: Drug metabolism and transporter properties of statins, in Focus on Statin Research, Wong BA, ed, Nova Science Publisher, 2006, NY

Gazzerio P, et al, Pharmacological Actions of Statins: A Critical Appraisal in the Management of Cancer, *Pharmacol. Revs.*, 2012, 64, 102.

Goosen TC, et al, Atorvastatin Glucuronidation Is Minimally and Nonselectively Inhibited by the Fibrates Gemfibrozil, Fenofibrate, and Fenofibric Acid, *Drug Metab. Dispos.*, 2007, 35, 1315.

Hoffmann M, Nowosielski M, DFT study on hydroxy acid–lactone interconversion of statins: the case of atorvastatin, *Org. Biomol. Chem.*, 2008, 6, 3527.

Jacobson W, et al, Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-coa reductase inhibitor atorvastatin, *Drug Metab. Dispos.*, 2000, 28, 1369.

Janagiraman S, et al, Electrochemical Determination of Pitavastatin Calcium As Bulk Drug by Voltammetry Techniques, *IJRSE, Int. J. Innov. Res. Sci. Engin.*, ISSN (Online) 2347-3207.

Jemal M, Ouyang Z, Chen BC, Teitz D, Quantitation of the acid and lactone forms of atorvastatin and its biotransformation products in human serum by high-performance liquid chromatography with electrospray tandem mass spectrometry, *Rapid Commun. Mass Spectrom.*, 1999, 13, 1003.

Kalliokoski A, Niemi M, Impact of OATP transporters on pharmacokinetics, *Br. J. Pharmacol.*, 2009, 158, 693.

Kobayashi M, Kagawa T, Takano R, Itagaki S, Hirano T, Iseki K, Effect of medium pH on the cytotoxicity of hydrophilic statins, *J. Pharm. Sci.*, 2007, 10, 332.

Kuriyan J, Kornforti D, Wemmer B, *The Molecules of Life*, Ch. 12, 2009, Garland Publishers.

Kantola T, Kivisto KT, Neuvonen PJ, Effect of itraconazole on the pharmacokinetics of atorvastatin, *Clin. Pharmacol. Ther.* 1998, 64, 58.

Kantola T, Kivisto KT, Neuvonen PJ, Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations, *Clin. Pharmacol. Ther.* 1998, 64, 177.

Koenen A, Kroemer HK, Grube M, Schwabedissen HEM, Current Understanding of Hepatic and Intestinal OATP-mediated Drug-drug Interactions, *Expert Rev. Clin. Pharmacol.*, 2011, 4, 729.

Kimoto E, et al, Characterization of Digoxin Uptake in Sandwich-Cultured Human Hepatocytes, *Drug Metab. Dispos.*, 2011, 39, 47.

Kunzl M, Wasinger C, Hohenegger M, Statins role in cancer prevention and development-recent meta-analyses, *World J. Pharmacol.*, 2013, 2, 100.

Kuypers FA, in *Medical Cell Biology*, 3rd ed, ed. Goodman SR, Elsevier, London 2008.

Lau YY, Huang Y, Frassetto L, Benet LZ, Effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers, *Clin. Pharmacol. Ther.*, 2007, 81, 194.

Law CJ, et al, Kinetic Evidence Is Consistent with the Rocker-Switch Mechanism of Membrane Transport by GlpT, *Biochemistry* 2007, 46, 12190.

Li J, et al, The Role of a Basolateral Transporter in Rosuvastatin Transport and Its Interplay with Apical Breast Cancer Resistance Protein in Polarized Cell Monolayer Systems, *Drug Metab. Dispos.*, 2012, 40, 2102.

Li N, et al, Use of a Novel Cell-based Model to Predict Hepatic Clearance of Statins: *In Vitro* to *In Vivo* Correlation, Corning, Presented at the 19th North American ISSX meeting, San Francisco, CA, Oct 19 - Oct 23, 2014.

Lodish H, Berk A, Zipursky SL, et al, Molecular Cell Biology. 5th edition, 2004, W.H. Freeman, New York.

Marenich AV, Cramer CJ, Truhlar DJ, Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions, *J. Phys. Chem B*, 2009, 113, 6378.

Margaritis EA, Channon KM, Antoniadis C, Statins as Regulators of Redox State in the Vascular Endothelium: Beyond Lipid Lowering, *Antioxidants & Redox Signaling*, 2014, 20, 8.

Mayo Clinic 2015 <http://www.mayoclinic.org/diseases-conditions/high-blood-cholesterol/in-depth/statin-side-effects/art-20046013>

Medsafe 2014, <http://www.medsafe.govt.nz/profs/PUArticles/March2014StatinsAndCYPInteractions.htm>

Macwan J, Model-Based Approaches to Characterize Clinical Pharmacokinetics of Atorvastatin and Rosuvastatin in Disease State (2013). Open Access Dissertations. Paper 28. http://digitalcommons.uri.edu/oa_diss/28.

Narwal N, Akhlaghi F, Asberg A, Hermann M, Rosenbaum SE, Development of a population pharmacokinetic model for atorvastatin acid and its lactone metabolite, *Clin. Pharmacokinet.* 2010, 49, 693.

Nicholls DG, Ferguson SJ, *Bioenergetics 2* (2nd ed.). 1992, San Diego, Academic Press, ISBN 9780125181242.

Nigovic B, Electrochemical properties and square-wave voltammetric determination of pravastatin, *Analytical and Bioanalytical Chemistry*, 2006, Volume 384, 431.

Nigovic B, Pavkovic I, Preconcentration of the lipid-lowering drug lovastatin at a hanging mercury drop electrode surface, *J. Anal. Chem.*, 2009, 64, 304.

Neuvonen PJ, Drug interactions with HMG-CoA reductase inhibitors (statins): the importance of CYP enzymes, transporters and pharmacogenetics, *Curr. Opin. Investig. Drugs.*, 2010, 11, 323.

Neuvonen PJ, Jalava KM, Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid, *Clin. Pharmacol. Ther.* 1996, 60, 54.

Oswald S, In vivo probes of drug transporters, *Drug Transporters* M Fromm & RB Kim eds, *Handbook of Experimental Pharmacology*, 2012, Springer Berlin 2012, p 403.

Perola E, Charifson PS, Conformational analysis of druglike molecules bound to proteins: an extensive study of ligand reorganization upon binding. *J. Med. Chem.* 2004, 47, 2499.

Prueksaritanont T, et al, Glucuronidation of statins in animals and humans: a novel mechanism of statin lactonization, *Drug Metab Dispos.* 2002, 5, 505.

Rayne S, Forest K, Accuracy of computational solvation free energies for neutral and ionic compounds: Dependence on level of theory and solvent model, *Nature Proceedings*, (2010) <http://dx.doi.org/10.1038/npre.2010.4864.1>

Rizzo RC, Aynechi T, Case DA, Kuntz ID, Estimation of Absolute Free Energies of Hydration Using Continuum Methods: Accuracy of Partial Charge Models and Optimization of Nonpolar Contributions, *J. Chem. Theory Comput.* 2006, 2, 128.

Rodrigues A, Efflux and uptake transporters as determinants of statin response, *Expert Opinion Drug Metab. Toxicol.* 2010, 6, 621.

Roth M, Obaidat A, Hagenbuch B, OATPs, OATs and OCTs: the organic anion and cation transporters of the *SLCO* and *SLC22A* gene superfamilies, *Br. J. Pharmacol.*, 2012, 165,1260.

Saito Y, Pitavastatin: an overview, *Atheroscler. Suppl.*, 2011, 12, 271.

Sakaeda T, et al, Effects of acid and lactone forms of eight HMGCoA-reductase inhibitors on CYP-mediated metabolism and MDRI-mediated transport, *Pharm, Res.*, 2006, 23, 506. Statin inhibitory effects were tested on paclitaxel 3-hydroxylation by CYP3A4/5.

Schneck DW, et al, The effect of gemfibrozil on the pharmacokinetics of rosuvastatin. *Clinic, Pharmacol. Therap.*, 2004, 75, 455.

Sharma P, Butters CJ, Smith V, Elsby R, Surry D, Prediction of the in vivo OATP1B1-mediated drug-drug interaction potential of an investigational drug against a range of statins, *Eur. J. Pharm. Sci.*, 2012, 47, 244.

Shishehbor MH, et al, Statins Promote Potent Systemic Antioxidant Effects Through Specific Inflammatory Pathways, *Circulation* 2003, 108, 426.

Shitara Y, Hori T, Sugitama Y, *Europ. J. Pharm. Sci.*, 2006, 27, 425.

Shitara Y, et al, Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles in hepatic clearance and intestinal absorption, *Biopharm. Drug Dispos.* 2013, 34, 45.

Volpe D, et al, Use of Caco-2 knockdown cells to investigate transporter-mediated efflux of statins, *AAPS* 2010, poster T3371.

Simonson SG, et al, Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporin. *Clin. Pharmacol. Therap.*, 2004, 76, 167.

Sissung TM, et al, Transporter pharmacogenetics: transporter polymorphisms after normal physiology, diseases, and pharmacotherapy, *Discovery Medicine*, January 17, 2012.

Skottheim IB, Gedde-Dahl A, Hejazifar S, Hoel K, Asberg A, Statin induced myotoxicity: the lactone forms are more potent than the acid forms in human skeletal muscle cells in vitro, *Eur. J. Pharm. Sci.* 2008, 33, 317.

Topol IA, et al, Experimental determination and calculations of redox potential descriptors of compounds directed against retroviral zinc fingers: Implications for rational drug design, *Protein Science*, 2001, 10, 1434.

Tornio A, Pasanen MK, Laitila J, Neuvonen PJ, Backman JT, Comparison of 3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) Reductase Inhibitors (Statins) as Inhibitors of Cytochrome P450 2C8, *Basic & Clinical Pharmacology & Toxicology*, 2005, 97, 104.

N. Treitel N, Shenhar R, Aprahamian I, Sheradsky R, Rabinovitz M, Calculations of PAH anions: When are diffuse functions necessary? *Phys. Chem. Chem. Phys.* 2004, 6, 1113.

Watanabe T, et al, Investigation of the Rate-Determining Process in the Hepatic Elimination of HMG-CoA Reductase Inhibitors in Rats and Humans, *Drug Metab. Dispos.*, 2010, 38, 215.

Whitfield LR, Porcari AR, Alvey C, Abel R, Bullen W, Hartman D, Effect of gemfibrozil and fenofibrate on the pharmacokinetics of atorvastatin, *J. Clin. Pharmacol.*, 2011, 51, 378

Yan JL, Determination of Fluvastatin Sodium by Differential Pulse Voltammetry, *Pakis. J. Biol. Sci.*, 2006, 9, 2156.