Anti-cancer properties of “hydrophilic and hydrophobic” statins and cyclin-dependent kinases

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Highlights:
• Statins in vitro and in vivo anti-cancer efficacy
• Statin-HMG-CoA reductase inhibition
• Statin cellular influx transport
• Quantitative structure activity relationships
• Statins, cyclin-dependent kinases, cell cycle progression

Abstract

The general QSAR equation: Transport or Inhibition = $\Delta G_{\text{desolvation}} + \Delta G_{\text{lipophilicity}} + \text{Dipole Moment} + \text{Molecular Volume}$ is shown to apply to statin inhibition of HMG-CoA reductase, passive and active statin cellular transport, the inhibition of growth of pancreatic cancer cells, and the survival times of mice. The equation also applies to the inhibition of cyclin-dependent kinases, which are known to be involved with statins in affecting the progression of tumour cell cycles.

The common clinical practice of using “hydrophilic” and “hydrophobic” statins as being arbiters of anti-cancer efficacy is clearly wrong. Efficacy of statins is a function of how well desolvation, lipophilicity, dipole moment and molecular volume of individual statins affect the many pathways involved in their anti-cancer activities.

Abbreviations
$\Delta G_{\text{desolvation}}$: free energy of water desolvation, $\Delta G_{\text{lipophilicity}}$: free energy of lipophilicity or hydrophobicity, DM: dipole moment, $R^2$: multiple correlation coefficient, $F$: the $F$ test of significance, SE: standard errors for the estimates, $SE(\Delta G_{\text{desolvation}})$: standard errors of $\Delta G_{\text{desolvation}}$, $SE(\Delta G_{\text{lipophilicity}})$: standard errors of $\Delta G_{\text{lipophilicity}}$, standard errors of $\Delta G_{\text{lipophilicity}}$, SE: standard errors for dipole moments, SE (Molecular Volume): standard errors for molecular volumes as calculated from “t” distribution statistics.

Introduction

(a) Statins anti-cancer properties

Statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are currently the most commonly prescribed drug class in the world. Statins are used for primary and secondary prevention of cardiovascular diseases. Besides cholesterol reduction, pre-clinical studies have shown that statins may exert anti-neoplastic effects, through both HMG-
CoA reductase-dependent and HMG-CoA reductase-independent pathways. By competitive inhibition of HMG-CoA reductase in the mevalonate cycle, (Figure 1) statins prevent a variety of pathways that lead to the inhibition of tumour cell growth, metastases, angiogenesis and apoptosis. Statins are known to have an effect on the cell cycle particularly on the cyclin-dependent kinases (CDK) that regulate the cell cycle.¹⁻⁶ (Figure 2)

It is known that lovastatin at a concentration of 5 μM induces breast cancer cells to arrest in the G1 phase by inhibiting the proteasome which, in turn, leads to the accumulation of inhibitors of cyclin-dependent kinases (CDKI), such as p21<sup>WAF1/CIP1</sup> and p27. Lovastatin lactone is responsible for the inhibition of the proteasome and thus inhibition of p21<sup>WAF1/CIP1</sup> and p27 degradation. It has been proven that mevalonate restored and increased the activity of proteasome, which caused degradation of CDKI and facilitated entry into the next phase of the cell cycle.⁷

There are very few agents available to prevent the onset of cancers. Statins have been shown to be safe in humans and widely used clinically for lowering cholesterol levels. So, if they prove to have potent anti-cancer effects, it will be easy to adapt them for prevention and therapy of cancers in humans. HMG-CoA reductase inhibition by statins leads to anti-cell-cycle inhibition of cyclin-dependent kinases and G1 to S progression, as well as additional inhibition of other signal transducers.

However while there is evidence supporting the positive role for statins in preventing cancer, there are some contrary views that statins may in fact cause cancer.⁸ A major difficulty in clarifying the role of statins regarding their anti-cancer behaviour in clinical trials is that there are very significant confounding issues that cloud the interpretation of clinical outcomes, such as age, many patients having existing cardiovascular disease, diabetes mellitus, being users of aspirin, metformin and other concomitant drugs, smokers, statin users being health conscious and more compliant with cancer screening leading to early cancer detection and treatment, translating improved survival etc.³
Figure 1. Schematic diagram of inhibition of mevalonate pathway by statins, and anti-tumour effects of statins

Figure 2. Schematic diagram showing relationships amongst the various cell phases, cyclins, cyclin-dependent kinases (CDK) and how statins disrupt expression/activation of cell cycle genes/proteins. Asterisks (*) indicate not in prostate cancer cells, arrows pointing down (↓) indicate decrease, and arrows pointing up (↑) indicate increase. X inhibition. (Adapted from Matusewicz 2015)

Two recent studies indicate that statin use was associated with an overall 22% reduction in deaths from breast, ovarian, colorectal, digestive cancer types (but not lung cancer) in a large number of post-menopausal women over 15 years and a 55% reduction in deaths from bone/connective tissue cancers. A study in a large number of men looked at statin use together with the anti-diabetes medication metformin and found a 40% reduction in prostate cancer mortality, with the effect more pronounced in men with obesity/metabolic syndrome.
There are several mechanisms in cancer cells that are activated by statins, some of them leading to cell death. \(^1\)-\(^5\), \(^1\)-\(^4\), \(^1\)-\(^1\) Statins exert different anti-cancer effects depending on:

- **cell line and tumour type:** clinically statins are considered to be effective against ovarian, colorectal, digestive cancer types (but not lung cancer) in post-menopausal women over 15 years and a 55% reduction in deaths from bone/connective tissue cancers. \(^9\) In men, statin use together with the anti-diabetes medication metformin, a 40% reduction in prostate cancer mortality was found. The US National Cancer Institute is investigating the role of statins in colorectal and melanoma skin cancers.

- **statin species and concentration:** statins can exist in three possible forms, the neutral acid, the neutral lactone, and the anionic acid form, with concentrations depending on pH, and whether some statins are administered as the lactone pro-drug eg lovastatin and simvastatin. However small concentrations of the three forms can exist in equilibrium around the physiological pH.

- **the type of statin ("hydrophilic" or "hydrophobic")** may have a large effect on the transport of statins across the cell membrane. The form of the statins has a large effect on the hydrophobic and hydrophilic properties of statins, with the neutral acid and lactones being more hydrophobic than the charged anionic form which are more hydrophilic.

- **duration of exposure of tumour cells to statins,** such as statin influx into cells and statin efflux out of the cell, particularly by multi-drug resistant transporters, and metabolic clearance of statins

- **by inhibition of the synthesis of cholesterol,** statins may destabilize the cell membrane by reducing myocyte membrane cholesterol. Instability of the cell membrane could lead to cell lysis and myopathy. Reduction of cholesterol could affect transport into and out of the cell, possibly by changing the fluidity of the lipid rafts in the cell membrane.

- **statins may change the arrangement of the transporter OATP1. OATP1B1 is the major transporter in hepatocytes.**

- **statins may affect the localization of HMG-CoA reductase**

- **statins can inhibit the cell cycle by affecting both expression and activity of proteins involved in cell-cycle progression such as cyclins, cyclin-dependent kinases (CDK), and/or inhibitors of CDK.**

- **statins may induce apoptosis by both intrinsic and extrinsic pathways**

- **statin treatment may lead to changes in molecular pathways dependent on the EGF receptor, mainly via inhibition of isoprenoid synthesis**

- **statins may induce conformational changes in glucose transporter proteins**

- **statins will be used in combination with other anti-cancer drugs in chemotherapeutic regimes**

In summary, it is clear that the anti-cancer efficacy of statins is dependent on many cellular pathways and the physico-chemical and metabolic properties of the various statins.

### (b) Hydrophobic versus hydrophilic statins: anti-cancer efficacy

Much of the focus on the preventative in vivo anti-tumour effects of statins has centred on whether there is a positive anti-neoplastic effect that can be distinguished from the many confounding comorbidity effects. Little attention has focussed on what properties of statins may be important for anti-tumour effectiveness. *The principal differentiator in the literature focuses on the “lipophilic” or “hydrophobic” statins as being effective, and the “hydrophilic” statins as being less effective.* \(^1\), \(^5\)-\(^2\) Hydrophilic statins are considered to be pravastatin and rosuvastatin, while lipophilic or hydrophobic statins are considered to be
cerivastatin, simvastatin, lovastatin, fluvastatin, atorvastatin, and pitavastatin. Hydrophilic statins are thought to mainly accumulate in the liver through uptake by the hepatic transporter OATP1B1, whereas hydrophobic statins are thought to be widely distributed in various tissues as they are considered to be able to passive permeate through cell membranes, unlike the hydrophilic statins. Hydrophilic statins are mainly metabolized by cytochromes, whereas pravastatin and rosuvastatin are not metabolically cleared by cytochromes.

However the notion that hydrophilic statins cannot easily pass through cell membranes (unless they are actively transported by hepatic transporters such as OATP1B1) unlike the hydrophobic statins is not supported in studies of whether statins can pass through the blood brain barrier (BBB), the most physiologically tight barrier in the body. There is evidence that hydrophilic and hydrophobic statins can pass through the BBB. Simvastatin, lovastatin and pravastatin (supposedly hydrophilic) have been detected in the cerebral cortex, with brain levels of the three statins reflecting their lipophilicity: simvastatin levels >lovastatin levels> pravastatin levels. Pravastatin has been shown to be transported into cells by organic anionic transporters (OATs) and these transporters are expressed in the brain. Another transporter that transports statin acids into cells is the monocarboxylic acid transporter, MCT4. In a study of simvastatin and lovastatin lactones and acids, it was concluded that the lactones were transported across the rat BBB by diffusion, whereas the acid forms were thought to be transported by MCT transporters.\(^{21-24}\)

Statins can exist in three forms, often in equilibrium depending on the physiological conditions, particularly pH: the neutral acid form, the charged anionic acid form, and the neutral lactone form. Simvastatin and lovastatin are administered as the lactone, which then undergo biotransformation to the acid and then the anionic form around the physiological pH 7 to act as inhibitors of HMG-CoA reductase. The role of the lactone form during various metabolic processes, including cytochrome clearance, has often been under recognized,\(^{25}\) but statin lactones play a critical role in muscle myopathy and related unwanted side effects of statins. The form of the statins plays a crucial role in transport of statins across cell membranes, including the fact that the simvastatin and lovastatin lactones are the actual species transported across the BBB.\(^{29}\)

The notion that the anti-cancer ability of statins can be differentiated into cell membrane permeable hydrophobic and non-permeable hydrophilic statins is clearly oversimplified and largely incorrect. The notion that statins can be either hydrophilic or hydrophobic is also misleading and incorrect, since at a molecular level statins have both hydrophilic and hydrophobic (lipophilic) areas on the molecular surface exposed to water (or proteins when bound to that protein). The much used measure of lipophilicity logP for unionized drugs (or logD for ionized drugs) is based on partitioning of a drug between water and n-octanol, with n-octanol representing the lipophilic phase. However, n-octanol contains 2.8M water content, which can have a large influence on dissolution into the lipophilic phase, especially for drugs that are not very water soluble (most drugs). Hence logP or theoretical methods based on logP can be a misleading criterion for drug lipophilicity or hydrophobicity.\(^{25-29}\)

The form of the statin species has a large effect on the hydrophilic or hydrophobic properties of the statins, and all three acid, acid anion and lactone forms can be present in varying quantities in equilibrium around the physiological pH level.

(c) Application of QSAR model to predict statin anti-cancer behaviour
This study will use a model (general QSAR equation) which has been successfully applied to various transport and drug-protein binding processes. A common model for both processes based on 4 molecular physico-chemical properties of the prospective drugs has been previously applied to passive and facilitated diffusion, and active organic anion transporter drug membrane transport, statins-HMG-CoA reductase inhibition, some competitive statin-CYP enzyme binding processes, tyrosine kinase inhibitors, HIV-1 protease inhibitors, and cyclin-dependent kinases. The general QSAR equation also applies to the active competitive transport of these tyrosine kinase inhibitors by the hOCT3, OATP1A2 and OCT1 transporters. The model comprises four main properties: (a) desolvation energy in water (b) lipophilicity or hydrophobicity based on the solvation energy in a hydrophobic solvent such as n-octane or n-octanol; lipophilicity being a measure of how well a drug can interact with lipophilic cell membrane bilayers, and hydrophobicity being a measure of non-polar interaction between a drug and the hydrophobic sectors of a protein (c) dipole moment in water, as a measure of the polar attraction between the drug and its receptor target or cell membrane (d) the molecular volume of the drug in water as a measure of how well the prospective drug fits into the cavity of the target receptor protein or active protein transporter, or how well a drug can passively diffuse through a cell membrane. The basic model is shown in the general QSAR equation.

\[
\text{Transport or Inhibitory Binding} = \Delta G_{\text{desolvation}} + \Delta G_{\text{lipophilicity}} + \text{Dipole Moment} + \text{Molecular Volume}
\]

Results

(a) Inhibitory binding of statins to HMG-CoA reductase

Corsini et al\textsuperscript{30,31} have studied the inhibition of HMG-CoA reductase activity \(K_i\) (nM) by statin acid anions. These data show reasonable correlations with the general QSAR equation.

Inhibition of rat microsomal HMG-CoA reductase activity \(K_i\) (nM) by statin acid anions\textsuperscript{30} [Corsini 1995]: rosuvastatin 12.0, pitavastatin 1.7, pravastatin 2.3, fluvastatin 0.3, atorvastatin 1.7, cerivastatin 1.3, lovastatin 0.6, simvastatin 0.12, mevastatin 1.4 Rosuvastatin and mevastatin omitted as outliers

<table>
<thead>
<tr>
<th>(K_i)</th>
<th>(0.002\Delta G_{\text{desolvation}} + 0.046\Delta G_{\text{lipophilicity}} + 0.224\text{ Dipole Moment} + 0.33\text{ Molecular Volume} - 17.79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rosuvastatin</td>
<td>(0.002\times12.0 + 0.046\times0.3 + 0.224\times1.7 + 0.33\times12.0 - 17.79)</td>
</tr>
<tr>
<td>pitavastatin</td>
<td>(0.002\times1.7 + 0.046\times0.3 + 0.224\times1.3 + 0.33\times1.7 - 17.79)</td>
</tr>
<tr>
<td>pravastatin</td>
<td>(0.002\times2.3 + 0.046\times0.3 + 0.224\times0.6 + 0.33\times2.3 - 17.79)</td>
</tr>
<tr>
<td>fluvastatin</td>
<td>(0.002\times0.3 + 0.046\times0.3 + 0.224\times0.12 + 0.33\times0.3 - 17.79)</td>
</tr>
<tr>
<td>atorvastatin</td>
<td>(0.002\times1.7 + 0.046\times0.3 + 0.224\times1.4 + 0.33\times1.7 - 17.79)</td>
</tr>
<tr>
<td>cerivastatin</td>
<td>(0.002\times1.3 + 0.046\times0.3 + 0.224\times1.4 + 0.33\times1.3 - 17.79)</td>
</tr>
<tr>
<td>lovastatin</td>
<td>(0.002\times0.6 + 0.046\times0.3 + 0.224\times0.12 + 0.33\times0.6 - 17.79)</td>
</tr>
<tr>
<td>simvastatin</td>
<td>(0.002\times0.12 + 0.046\times0.3 + 0.224\times0.12 + 0.33\times0.12 - 17.79)</td>
</tr>
<tr>
<td>mevastatin</td>
<td>(0.002\times1.4 + 0.046\times0.3 + 0.224\times0.12 + 0.33\times1.4 - 17.79)</td>
</tr>
</tbody>
</table>

Equations 1 is highly significant, and indicates the most sensitive binding variable is the dipole moment, which are high for the anionic species. The desolvation energy, lipophilicity and molecular volume of the statins are of about equal sensitivity. Molar Volumes have been multiplied by 0.1 to give magnitude range similar to the magnitude ranges of other three independent variables in applying the general QSAR equation. Rosuvastatin is a very large outlier from the correlations, with mevastatin also a significant outlier. Rosuvastatin activity is sourced from a different study [31] than the other statin activity data from the one study.[30] Inclusion of mevastatin in eq 1 still gives a good correlation, \(R^2 0.877, F\)
significance 0.100 but it is clear that eq 1 is a stronger correlation if mevastatin is treated as an outlier. Correlations of activity were tested against the statin acids, but the correlations were poor, indicating that the anionic species which are the dominant species at physiological pH, are the likely inhibitory species.

(b) Application of the general QSAR model to passive and active transport of statins across cell membranes

(b)(1) Passive transport of statins

A passive diffusion permeation model for atorvastatin, fluvastatin, cerivastatin, pravastatin, pitastatin, lovastatin, rosuvastatin statins based on passive permeation diffusion data for the Corning Transporto cells control cells  has been developed. The best fit of the passive diffusion rate was for the lactone form, where equation 2 shows a positive correlation with the desolvation free energy in water, dipole moment and molecular volume, and a negative correlation with the lipophilicity.

Equation 2a which shows the passive diffusion rates for statin lactones in rat hepatocytes can be compared directly with eq 2 showing the in vitro passive diffusion rates for the same lactones. It can be seen that the relative magnitude and signs of the coefficients are similar (though the in vitro eq 2 is ca 10 times more sensitive than the in vivo eq 2a), with the exception of the dipole moment which is relatively much larger in the rat hepatocyte than the in vitro case.

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. This data has been analysed in equations 4 and 5. Equation 4 (lactone form) is more statistically robust than equation 5 (acid anion form) which suggests 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 $K_m$ 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 4 and 5 are 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.

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 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.
For the statin lactones: Corning Transporto cells,\(^\text{32}\) [Li 2014]  
\[
\text{Passive diffusion rate} = -2.96 \Delta G_{\text{desolvation}} + 7.51 \Delta G_{\text{lipophilicity}} + 2.26 \text{ Dipole Moment} + 11.1 \text{ Molecular Volume} - 143.53
\]
Where \(R^2 = 0.997\), SEE = 4.17, SE(\(\Delta G_{\text{desolvation}}\)) = 0.30, SE(\(\Delta G_{\text{lipophilicity}}\)) = 1.03, SE(Dipole Moment) = 0.68 SE(Molecular Volume) = 0.12, F=80.91, Significance=0.082, no=6

For the statin lactones: rat hepatocytes,\(^\text{33}\) [Shitara 2013]  
\[
\text{Passive diffusion rate} = -0.38 \Delta G_{\text{desolvation}} + 1.23 \Delta G_{\text{lipophilicity}} + 2.90 \text{ Dipole Moment} + 1.5 \text{ Molecular Volume} - 25.92
\]
Where \(R^2 = 0.961\), SEE = 4.97, SE(\(\Delta G_{\text{desolvation}}\)) = 0.16, SE(\(\Delta G_{\text{lipophilicity}}\)) = 1.23, SE(Dipole Moment) = 0.81 SE(Molecular Volume) = 0.14, F=6.141, Significance=0.290, no=6

Eq 2 and eq 2a are similar, indicative of similar (probably facilitated) diffusion processes.

For the statin acids: Corning Transporto cells,\(^\text{32}\) [Li 2014]  
\[
\text{Passive diffusion rate} = -1.05 \Delta G_{\text{desolvation}} + 10.47 \Delta G_{\text{lipophilicity}} + 3.54 \text{ Dipole Moment} + 3.3 \text{ Molecular Volume} + 98.87
\]
Where \(R^2 = 0.881\), SEE = 20.06, SE(\(\Delta G_{\text{desolvation}}\)) = 2.69, SE(\(\Delta G_{\text{lipophilicity}}\)) = 3.73, SE(Dipole Moment) = 4.83, SE(Molecular Volume) = 0.16, F=3.21, Significance=0.18, n=8

For the statin anions: Corning Transporto cells,\(^\text{32}\) [Li 2014] excluding cerivastatin outlier  
\[
\text{Passive diffusion rate} = 0.66 \Delta G_{\text{desolvation}} + 0.29 \Delta G_{\text{lipophilicity}} - 0.95 \text{ Dipole Moment} - 2.8 \text{ Molecular Volume} + 97.45
\]
Where \(R^2 = 0.919\), SEE = 8.95, SE(\(\Delta G_{\text{desolvation}}\)) = 0.54, SE(\(\Delta G_{\text{lipophilicity}}\)) = 0.32, SE(Dipole Moment) = 1.37, SE(Molecular Volume) = 0.22, F=5.69, Significance=0.155, no=7

(b)(2) Active transport of statins

For the active transport of statin lactones by OATP transporter, rat hepatocytes,\(^\text{33}\) [Shitara 2013]: Equation 4  
\[
K_m = -0.33 \Delta G_{\text{desolvation}} + 2.26 \Delta G_{\text{lipophilicity}} - 3.77 \text{ Dipole Moment} - 0.3 \text{ Molecular Volume} + 96.3
\]
Where \(R^2 = 0.972\), SEE = 5.40, SE(\(\Delta G_{\text{desolvation}}\)) = 0.39, SE(\(\Delta G_{\text{lipophilicity}}\)) = 1.34, SE(Dipole Moment) = 1.88, SE(Molecular Volume) = 0.15, F=8.76, Significance=0.024, no=6

For the active transport of statin acid anions by OATP transporter, rat hepatocytes,\(^\text{33}\) [Shitara 2013]: Equation 5  
\[
K_m = 1.79 \Delta G_{\text{desolvation}} + 2.67 \Delta G_{\text{lipophilicity}} - 7.57 \text{ Dipole Moment} - 6.1 \text{ Molecular Volume} + 471.67
\]
Where \(R^2 = 0.949\), SEE = 7.35, SE(\(\Delta G_{\text{desolvation}}\)) = 1.22, SE(\(\Delta G_{\text{lipophilicity}}\)) = 1.96, SE(Dipole Moment) = 2.82, SE(Molecular Volume) = 0.19, F=4.61, Significance=0.33, n=6

The correlation for the active transport of statin acids by OATP transporter, rat hepatocytes,\(^\text{34}\) was very poor, suggesting that OATP transporters do not transport the acid species. Eq 4 and 5 are indicative of rat OATP transporters being active with both anion and lactone species.

(c) Anti-cancer activity of statins: in vitro and in vivo
An elegant and extensive study of the inhibitory effects of statins on the growth of in vitro pancreatic cancer cells and the in vivo effect on nude mice survival has been documented. These data have been analysed by applying the general QSAR equation, and the results are given in Table 1.

**Table 1.** The effect of statin properties on growth of pancreatic cancer cells and nude mice survival times

<table>
<thead>
<tr>
<th>Statin Properties</th>
<th>CAPAN-2 24 hours</th>
<th>CAPAN-2 48 hours</th>
<th>MiaPaCa-2 24 hours</th>
<th>MiaPaCa-2 48 hours</th>
<th>BxPe-3 24 hours</th>
<th>BxPe-3 48 hours</th>
<th>BxPe-3 72 hours</th>
<th>Mice Survival Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desolvation ΔG&lt;sub&gt;desolvation&lt;/sub&gt;</td>
<td>-1.3(0.5)</td>
<td>-1.6(0.8)</td>
<td>-1.4(0.3)</td>
<td>-1.0(0.2)</td>
<td>-1.3(0.8)</td>
<td>-1.4(0.4)</td>
<td>-0.7(0.6)</td>
<td>-3.5(1.8)</td>
</tr>
<tr>
<td>Lipophilicity ΔG&lt;sub&gt;lipophilicity&lt;/sub&gt;</td>
<td>-0.3(0.3)</td>
<td>0.3(0.4)</td>
<td>-0.3(0.2)</td>
<td>0.2(0.1)</td>
<td>-0.3(0.4)</td>
<td>0(0.2)</td>
<td>0.5(0.3)</td>
<td>-1.1(1.0)</td>
</tr>
<tr>
<td>Dipole Moment</td>
<td>-1.0(1.2)</td>
<td>1.6(1.8)</td>
<td>-1.1(0.8)</td>
<td>1.3(0.5)</td>
<td>-1.3(1.9)</td>
<td>0(1.1)</td>
<td>2.4(1.5)</td>
<td>-5.6(4.4)</td>
</tr>
<tr>
<td>Molecular Volume*</td>
<td>-2.5(2.0)</td>
<td>0.2(3.0)</td>
<td>-2.7(1.4)</td>
<td>1.1(0.8)</td>
<td>-2.2(3.1)</td>
<td>-1.1(1.7)</td>
<td>2.3(2.5)</td>
<td>-22.5(7.3)</td>
</tr>
<tr>
<td>R² F significance</td>
<td>0.823 0.320</td>
<td>0.862 0.256</td>
<td>0.980 0.038</td>
<td>0.927 0.139</td>
<td>0.697 0.510</td>
<td>0.911 0.169</td>
<td>0.831 0.300</td>
<td>0.896 0.196</td>
</tr>
</tbody>
</table>

**Footnotes:** Statin properties as per the general QSAR equation IC<sub>50</sub> (or survival times) = ΔG<sub>desolvation</sub> + ΔG<sub>lipophilicity</sub> + Dipole Moment + Molecular Volume. IC<sub>50</sub> (μM) for pancreatic cancer cell lines treated with statins and mice survival times (days) taken from Gbelkova 2008. The nude mice were xenotransplanted subcutaneously with human pancreatic adenocarcinoma cell line CAPAN-2. The values for the properties are the coefficients for the correlation equations derived from the general QSAR equation and the values in brackets are the standard errors for the coefficients. R² is the square of the multiple regression coefficient, F significance is the F test of significance for the multiple regression correlation equation. Molar Volumes multiplied by 0.1 to give magnitude range similar to the magnitude ranges of other three independent variables for comparative purposes.

Table 1 shows the analyses using equation 1 for the inhibition of growth of three pancreatic cancer cell lines as well as the mice survival times for seven statins, rosuvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, lovastatin and simvastatin (as acid anions). The statistical analyses are not strongly robust as there are insufficient experimental data points, but the patterns across the three cell lines and mice data are consistent across cell lines and time. The standard errors of the coefficients indicate the statistical precision. The relative changes across cell lines and with time as well as the live mice results are critical in trying to elucidate mechanistic factors from this study. Gbelkova has indicated that other possible statin species (such as lactones) are not involved, and the known pK<sub>a</sub> of the statins under the experimental conditions indicate that the anionic form of the free acids are the active statin species.

Examination of the results in Table 1 show consistent patterns for the three in vitro pancreatic cancer cell lines:

1. Desolvation, dipole moment and molecular volume of the statins are of similar importance in inhibiting the growth of the cells. Lipophilicity has a minor effect.
2. There is a clear time elapsed effect, particularly for the effect of dipole moment. The time effect is most clear for the BxPe-3 cells where the desolvation, lipophilicity and dipole moment influence become consistently more positive over time. This suggests that the overall reaction mechanism of inhibition is changing.
3. The nude mice survival times are reflective of the cell line results (in that the relative magnitudes of the coefficients for desolvation, lipophilicity and dipole moment to each other are similar to those from the cell line data) with the exception of the much greater
negative effect of molecular volume of the statins on mice survival times. This outcome suggests that transport of the statins to the mouse tumours, either by passive or active transport mechanisms, is a critical factor. Molecular volume has a strong influence on the passage of molecules through the cell membrane bilayer in passive permeation, as well as a major effect on active transport across cell membranes via transporters, as shown in eqs 2-4.

(d) Properties of cyclin-dependent kinases (CDK)

We have previously studied the inhibitory activity against CDK2/cycE by the series \((4\text{-}RO\text{-}1\text{H-pyrazolo}[3,4-b]\text{pyridin-5-yl})(\text{phenyl})\text{methanone}\) (Figure 3) where RO was the alkoxy group with R being \(n\)-propyl (IC\(_{50}\)=0.18 \(\mu\)M), \(n\)-butyl (0.11), 1-methylbutyl (0.24), 3-methylbutyl (0.18), \(n\)-pentyl (0.52), 2-hydroxyethyl (3.7), 2-dimethylaminoethyl (17), cyclohexyl (0.66), benzyl (0.28), phenyl (15), and \((4\text{-}RO\text{-}1\text{H-pyrazolo}[3,4-b]\text{pyridin-5-yl})(\text{phenyl-R})\text{methanone}\) where R was \(n\)-butyl and phenyl-R was 2,6-difluorophenyl, 2,6-difluoro-4-methyphenyl, 2,4,6-trimethylphenyl, 2-fluorophenyl and 2-methylphenyl.\(^{27}\)

![Figure 3](image)

**Figure 3.** CDK inhibitors, \((4\text{-}RO\text{-}1\text{H-pyrazolo}[3,4-b]\text{pyridin-5-yl})(\text{phenyl})\text{methanone}\) showing alkoxy \((R = n\text{-butyl})\) substituents, and \((4\text{-butoxy-1H-pyrazolo}[3,4-b]\text{pyridin-5-yl})(\text{phenyl-R})\text{methanone}\) illustrated with 2,6-difluoro-4-methyl substituents on the phenyl-R ring.

IC\(_{50}\) values (\(\mu\)M) for CDK2/cycE of the inhibitor series \((4\text{-}RO\text{-}1\text{H-pyrazolo}[3,4-b]\text{pyridin-5-yl})(\text{phenyl})\text{methanone}\) where RO is the alkoxy group with R being \(n\)-propyl (0.18), \(n\)-butyl (0.11), 1-methylbutyl (0.24), 3-methylbutyl (0.18), \(n\)-pentyl (0.52), 2-hydroxyethyl (3.7), 2-dimethylaminoethyl (17), cyclohexyl (0.66), benzyl (0.28), phenyl (15), and \((4\text{-}RO\text{-}1\text{H-pyrazolo}[3,4-b]\text{pyridin-5-yl})(\text{phenyl-R})\text{methanone}\) where R was \(n\)-butyl and phenyl-R was \(R\) being \(n\)-butyl with phenyl-R being 2,6-difluorophenyl (0.064), 2,6-difluoro-4-methyphenyl (0.036), 2,6-difluoro-4-methoxyphenyl (0.022), 2,6-difluoro-4-chlorophenyl (0.027), 2,6-difluoro-4-bromophenyl (0.020), 2-fluorophenyl (0.036) and 2-methylphenyl (0.073). R = phenyl and 2-dimethylaminoethyl omitted as outliers.  

\[
\text{Eq 6} \quad \text{IC}_{50} = 0.42 \Delta G_{\text{desolvation}} + 0.27 \Delta G_{\text{lipophilicity}} - 0.05 \text{ Dipole Moment} + 0.06 \text{ Molecular Volume} - 2.57
\]

Where \(R^2 = 0.753, \text{SEE} = 0.52, \text{SE}(\Delta G_{\text{desolvation}}) = 0.08, \text{SE}(\Delta G_{\text{lipophilicity}}) = 0.19, \text{SE}(\text{Dipole Moment}) = 0.12, \text{SE}(\text{Molecular Volume}) = 0.006, F=8.38 \text{ Significance} F = 0.002, n=16\)

Similar correlations to eq 6 were found for CDK1/cycB and CDK4/cycD.

**Discussion**

It is clear from the literature that the anti-cancer properties of statins are multi-causal, and that there are many inter-linked pathways that contribute to the overall efficacy of statins. It is implausible that all these pathways can be identified or separate mechanisms portrayed.
However, if a “universal” QSAR can be identified that may apply to all or most of these pathways, then some degree of predictability may be possible. It is postulated that the general QSAR equation may be applicable, since it appears to apply to many binding and cellular transport activities for a wide range of drugs.

Critical factors that govern the statin efficacy include bioavailability, the potential equilibria that exists amongst the acid, acid anion and lactone forms of the statins in the circulatory system at the physiological pH and within the tumour cell at the cystolic pH (usually slightly acidic), the rate that the various forms of the statins can be transported across the cell membrane in tumour tissue (influx), the rate of efflux out of tumour cells, metabolic clearance rates, followed by the rate of the HMG-CoA reductase inhibition by the statins, and the rates of the various other anti-cancer inducing pathways described in Figure 1. A logical and first order approach to the plethora of processes involved is to treat the influx rate into the tumour cell as the overall rate determining step that governs all other sequential processes that occur within the cell, including efflux. The second fastest rate determining step should be the rate that statins inhibit HMG-CoA reductase in tumour cells. If these two assumptions are predominantly correct, then the form of the general QSAR equation for these two steps should be a rough approximation of the overall process. Eq 1-5 are broadly consistent with this notion. Analysis of the Table 1 data suggests that the 24-72 hour cell data for the three cell lines changes and appears to start to mirror the mice survival results in that the relative magnitudes of the desolvation, lipophilicity and dipole moment coefficients are similar, with the exception of the much stronger dependence on molecular volume for the mice data. This result may indicate that transport of the statins into the tumour cells is the long term dominant determinant of anti-cancer efficacy.

The general QSAR equation has been shown to apply to inhibition of HMG-CoA reductase and the passive and active transport of statins, as shown in eq 1-5. It also applies to the inhibition of a wide range of cyclin-dependent kinases (eq 6), which is significant since it is known that there exists some relationship amongst statins, CDK and cell cycle progression.

The analysis of the effect of statin properties on growth of pancreatic cancer cells and nude mice survival times shown in Table 1 clearly show that the general QSAR equation can accurately describe how statins affect pancreatic cancer cell growth and mice survival times. The cell line data also shows a time dependency, which would be expected for multiple pathways that change in importance as a function of time.

Finally, the application of the general QSAR equation to statin anti-cancer efficacy clearly shows that the commonly used clinical distinction between “hydrophilic” and “hydrophobic” statins as being arbiters of anti-cancer efficacy is clearly wrong. Efficacy of statins is a function of how well desolvation, lipophilicity, dipole moment and molecular volume of individual statins affect the many pathways involved in their anti-cancer activities.

Conclusions

The general QSAR equation: Transport or Inhibition = ΔG_{desolvation} + ΔG_{lipophilicity} + Dipole Moment + Molecular Volume is shown to apply to statin inhibition of HMG-CoA reductase,
passive and active statin cellular transport, the inhibition of growth of pancreatic cancer cells, and the survival times of mice. The equation also applies to the inhibition of cyclin-dependent kinases, which are known to be involved with statins in affecting the progression of tumour cell cycles.

The common clinical practice of using “hydrophilic” and “hydrophobic” statins as being arbiters of anti-cancer efficacy is clearly wrong. Efficacy of statins is a function of how well desolvation, lipophilicity, dipole moment and molecular volume of individual statins affect the many pathways involved in their anti-cancer activities.

In the absence of better predictive methods, the general QSAR equation may serve as a useful predictor of overall statin anti-cancer efficacy and some of the many pathways that contribute to the overall efficacy.

### Table 2. Solvation energies, lipophilicities, dipole moments, molecular volumes of statins

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<tr>
<th>Statin Desolvation</th>
<th>Lipophilicity</th>
<th>Dipole Moment</th>
<th>Molecular Volume</th>
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<td>Energy Water kcal/mol</td>
<td>kcal/mol</td>
<td>D Water cm³/mol</td>
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<tr>
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**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.
Values in cm³/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.

**References**


[25] Fong, C.W. Statins in therapy: Cellular transport, side effects, drug-drug interactions and cytotoxicity - the unrecognized role of lactones, HAL Archives, 2016; hal-01185910v2,
[29] 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.
[35] All calculations were carried out using the Gaussian 09 package at the B3LYP/6-31 G*(6d, 7f) level of theory with optimised geometries in water, 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. 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. Adding diffuse functions to the 6-31G* basis set (ie 6-31**G**) had no significant effect on the solvation energies with a difference of less than 1% observed in solvents, which is within the literature error range for the IEFPCM/SMD solvent model. 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 $K_m$, $IC_{50}$ to develop the multiple regression equations have much higher uncertainties than the calculated molecular properties. The statistical analyses include the multiple correlation coefficient $R^2$, the F test of significance, standards errors for the estimates (SEE) and each of the variables SE($\Delta G_{\text{desolvation}}$), SE($\Delta G_{\text{lipophilicity}}$), SE(Dipole Moment), SE (Molecular Volume), SE(Molecular Volume), as calculated from “t” distribution statistics. Residual analysis was used to identify outliers. The solvation energies, lipophilicities, dipole moments, molecular volumes of the statins are shown in Table 2.

