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Drug discovery model using molecular orbital computations: tyrosine kinase inhibitors

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Keywords

QSAR model, tyrosine kinase inhibitors, multi-kinase inhibitors, kinase binding, active protein transporters, quantum mechanics

Abbreviations

TKI Tyrosine kinase inhibitors, MKI Multi-kinase inhibitors, CML chronic myeloid leukaemia, organic cation uptake transporter OTP, organic anion uptake transporter OATP, organic cation influx transporter OCT, SDF surface distribution function, RDF the water radial distribution function, $\Delta G_{\text{desolvation}}$ free energy of water desolvation, $\Delta G_{\text{lipophilicity}}$ free energy of lipophilicity or hydrophobicity, $\Delta G_{\text{desolv,CDS}}$ free energy of water desolvation of the cavitation dispersion solvent structure (CDS), $\Delta G_{\text{lipo,CDS}}$ free energy of lipophilicity or hydrophobicity for the CDS, DM dipole moment DM, SASA solvent accessible surface area, R^2 multiple correlation coefficient, F the F test of significance, SEE standards errors for the estimates, $SE(\Delta G_{\text{desolvation}})$ standard errors of $\Delta G_{\text{desolvation}}$, $SE(\Delta G_{\text{lipophilicity}})$, standard errors of $\Delta G_{\text{lipophilicity}}$, SE(Dipole Moment) standard errors for dipole moments, SE (Molecular Volume) standard errors for molecular volumes as calculated from "t" distribution statistics, QM quantum mechanics, DDR Discoidin domain receptor, PDGFR platelet-derived growth factor receptors

Abstract

It has been shown that equation 1 can be successfully applied to the competitive binding of a number of tyrosine kinase inhibitors and multi-kinase inhibitors to a range of kinase enzymes. Equation 1 also applies to the active competitive transport of these tyrosine kinase inhibitors by the hOCT3, OATP1A2 and OCT1 transporters. There is strong independent evidence from the literature that $\Delta G_{\text{desolvation}}$, $\Delta G_{\text{lipophilicity}}$, the dipole moment and molecular volume are good inherent indicators of the transport or binding ability of drugs.

Equation 1:

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

Or

$$\text{Transport or Binding} = \Delta G_{\text{desolv,CDS}} + \Delta G_{\text{lipo,CDS}} + \text{Dipole Moment} + \text{Molecular Volume}$$

A modified form of equation 1 using the free energy of water desolvation ($\Delta G_{\text{desolv,CDS}}$) and the lipophilicity free energy ($\Delta G_{\text{lipo,CDS}}$), where CDS represents the first solvation shell solvent properties, may be a good approximation of the drug approaching the entry of the protein receptor pocket or the surface of the protein transporter. Desolvation of water from the drug ($\Delta G_{\text{desolv,CDS}}$) before binding in the receptor pocket is required, and hydrophobic interactions between the drug and protein ($\Delta G_{\text{lipo,CDS}}$) is a positive contribution to binding.

Equation 1 or its modified form may be useful guides to drug discovery and design, particularly the allowing examination of the various species of a potential drug that may predominate at different pH levels, or by making changes to the molecular structure to predict binding or transport properties.

Objective: Develop a broadly applicable general model of membrane transport and drug-protein binding:

Introduction

Drug efficacy in the broadest sense can be described as the efficiency of the processes of drug delivery or transport to its intended target, followed by the drug binding or interacting with its target receptor. For an orally administered drug, these processes include intestinal solubility and the full range of ADMET (absorption, distribution, metabolism, excretion, and toxicity) processes which include transport across cellular membranes. In many cases, the target is a protein or enzyme, and drug binding within a receptor pocket is a crucial phase of the efficacy process. In many cases, there are multiple receptor pockets in the same enzyme, so unintended side effects may occur.

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 and some competitive statin-CYP enzyme binding processes. [1,2][Fong 2015, 2014] The model comprises four main properties: (a) desolvation energy in water (b) lipophilicity or hydrophobicity based on solvation energy in 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 diffuse through a cell membrane. The basic model is shown in **equation 1**.

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

The method uses quantum mechanical methods to compute each of the 4 independent variables for the drug and relies on multiple correlation with experimental values for transport or binding processes, preferably from the same source to minimize errors in experimentally determined values. Since sufficient experimental data are generally not available for a robust multi-variate statistical analysis, it is important that there are other physico-chemical studies that can verify the validity and appropriateness of using each of the four independent variables.

The application of equation 1 to membrane transport has shown that desolvation is a dominant feature for passive and facilitated diffusion, and organic anion transporter processes before the drug can enter the membrane or transporter. Lipophilicity or hydrophobicity, the dipole moment and molecular volume become important within the membrane or transporter. It can be seen that the passage of a drug through a lipophilic membrane, or within the interior of a transporter, is similar to the binding of a drug within a largely lipophilic pocket of an

enzyme. We have also shown that eq 1 fits the solubility of a wide range of drugs in water. [3][Fong, 2015]

A full quantum mechanical (QM) study of the binding of imatinib and nilotinib with the Bcr-ABL tyrosine kinase has shown that desolvation of the binding pocket and the inhibitor is required during the binding process in the enzyme pocket. Also the *total* hydrophobic or van der Waals interaction between the inhibitor and kinase is much larger than the electrostatic or coulombic interaction (from hydrogen bonding, polar bonding) between the inhibitor and kinase. [4][Fong 2015]

Water: Desolvation in binding pocket

In molecular dynamics studies of the binding of dasatinib to src-tyrosine kinase, it has been shown that almost complete desolvation of the binding pocket occurs before complex formation does occur. [5-7][Setny 2010, Mondial 2014, Shan 2011] As the ligand approaches the protein pocket, complete dewetting of pocket occurs (mainly enthalpy driven) even though the pocket could accommodate the first shell of hydration around ligand. Disorganized water in the cavity can have more entropy than in the bulk solvent & its release eliminates water fluctuation within the pocket, while the release of water from the hydrophobic environment within pocket to bulk water results in a gain of enthalpy. These studies are consistent with the QM study of imatinib and nilotinib binding in the Bcr-ABL tyrosine kinase pocket, where the calculation of drug-protein binding energy included complete desolvation of the drug and the inner area of the protein pocket immediately surrounding the bound drug before binding with the protein. [4][Fong 2015] These computational studies are supported by the analysis on a large set of proteins which showed that the average dielectric constant inside the protein is relatively low, about 6–7, and reaches a value of about 20–30 at the protein's surface. [8][Li 2013] Since bulk water has a dielectric constant of 78.3D, the environment surrounding an incoming drug changes from 78.3D in the bulk water solvent to ca. 20-30 at the opening of the protein pocket, to ca. 6-7 inside the pocket after binding. Thermodynamically, this transition must lead to energy intensive desolvation of the drug. In a study of the desolvation energies required for K^+ and Na^+ ions to bind with the carrier ionophores valinomycin or crown ethers, it was found that desolvation energies were the dominant factors governing binding of K^+ and Na^+ to the valinomycin. [9][Fong 2016]

Protein crystal structures (105 at atomic resolution $\leq 1.0 \text{ \AA}$) with a high level of hydration water were examined by calculating the experimental water-protein radial distribution function or surface distribution function (SDF) and the water radial distribution function (RDF). The first maximum at 2.75 \AA represents the centre of the inner layer of hydration water formed by hydrogen bonds between water and polar atoms of the protein. The second maximum at 3.65 \AA represents the outer layer of hydration water formed by water-water hydrogen bonds and van der Waals interactions between water and non-polar atoms of the protein forming clathrate-hydrate-like structures. The SDF describes the density of water as a function of the distance from the protein surface (the closest non-hydrogen atom). The RDF describes the density of water as a function of the distance from a particular water molecule. There was a significant difference in the water structure and water potentials between hydration water and bulk water in protein crystals. The first and second maxima of the RDF are at 2.75 and 4.5 \AA , respectively, the same distances as those of bulk water. However, the maxima/minima of the RDF of hydration water are higher/lower than those of bulk water, indicating that hydration water is denser and narrowly defined, likely due to stronger

interactions induced by the protein. [10] [Chen 2008] As the X-ray structures of various TKI-kinase complexes show polar bonding with tyrosine kinase proteins fall within the 2.75-4.5Å range, it appears then that it is the first or inner solvent shells of the TKI and kinases that are the important indicators for gauging the extent of desolvation that must occur for TKI-kinase complex binding to occur. The X-ray protein hydration results are consistent with desolvation of the protein pocket before binding by the inhibitor, as found in molecular mechanics studies for dasatinib-src-tyrosine kinase [5-7][Setny 2010, Mondial 2014, Shan 2011] or quantum mechanical studies of nilotinib- and imatinib-ABL tyrosine kinase. [4][Fong 2015]

Dipole moment: polar and non-polar interactions

The DM of a drug in water is a vector measure of the product of the magnitude of the positive and negative electrostatic charges on the molecule and the distance separating the charges. The DM is a measure of the overall polarity of the molecule. Drug molecules usually have a mixture of hydrophilic and hydrophobic groups, the proportion and distribution of which determines the overall DM. Reversible competitive inhibition of a proteins usually involves an intermolecular non-covalent interaction between an inhibitor and a protein receptor. These interactions can be electrostatic, π -effects, van der waals forces and hydrophobic effects. Hydrogen bonding is usually the strongest of the electrostatic interactions, followed by salt bridges, often of the order of 4-10 kcal/mol. π -effects, van der waals forces and hydrophobic effects are usually much smaller in magnitude, usually much less than ca. 1-2 kcal/mol. Hydrophobic interactions are not specific, but occur between non-polar hydrocarbon groups on the drug and receptor sites which tend to exclude water molecules in the binding region. Hydrophobic interactions are usually very small, but numerous, and can arise from the burying of hydrophobic surfaces during drug-receptor binding. The solvent accessible surface area (SASA) of the drug is commonly taken as a proxy of the extent of the hydrophobic effect, and the extent of this effect is thought to be between 5 and 45 cal/(Å² mol). 11-13][[Sharp 1991, Southall 2002, Snydera 2011] Olsson [14][2008] have examined the SCORPIO data base of published isothermal titration calorimetric results for a range of protein and small ligand interactions with changes in solvation (using the polar and non-polar solvent accessible surface area of the ligand and changes resulting from the protein-ligand complexation). Most interactions were enthalpy driven. The strongest correlation was between non-polar surface area burial upon complexation and the binding free energy, consistent with an entropy driven process (with $T\Delta S^\circ$ being about double ΔH°). However the free energy contribution per unit area buried was only about 30-50% of previous similar studies of transfer free energies of small ligands. The transfer of an overall polar drug like nilotinib, (13.1D) or a charged drug like imatinib ion (46.0D) from water with a dielectric constant of 78.3 to a nonpolar media with dielectric constant of ~3 (lipid bilayer) or 4 to 10 (interior of proteins) costs significant energy [15][Baldwin 2005]. Analysis on a large set of proteins shows that (a) the average dielectric constant inside the protein is relatively low, about 6-7, and reaches a value of about 20-30 at the protein's surface, and (b) high average local dielectric constant values are associated with charged residues while low dielectric constant values are assigned to the regions occupied by hydrophobic residues. [8][Li 2013] N-Octane has a dielectric constant of 1.94 and should be a good proxy for the interior of a protein.

One estimate gives a free energy gain of ~ 0.03 kcal/mol/Å² (buried polar surfaces giving up 0.1 kcal/mol/Å²), such that every 46 Å² of buried hydrophobic surface area (for example a Me group) gives a 10 increase in potency. [15][Baldwin 2005] Another estimate is that every methylene unit added to a hydrocarbon chain increases the free energy of transfer into water

by a fixed amount, by between 0.025 and 0.033 kcal/mol/Å² [12][Southall 2002] Hydrophobic interactions appear to be the dominant driving force for induced fit of receptors around drugs, and can also be described as hydrophobic induced fit collapse of a receptor around a drug. Charge-reinforced hydrogen bonds can contribute up to 3000-fold in binding, but the more common neutral-neutral hydrogen bonds may contribute anything between zero and 15-fold. So the more numerous hydrophobic interactions can easily dominate hydrogen bonding in drug receptor binding [Davis 1999, Baldwin 2005] It has also been shown that studies of the binding of drugs to biological receptors using molecular force fields cannot adequately incorporate hydrophobic interactions or conformational changes during binding, and can use *ad hoc* values of 0.02 to 0.04 kcal/mol/Å² for *all* types of atoms. [16][Davis 1999] A study of the binding of the imatinib ion and nilotinib with Bcr-ABL tyrosine kinase has shown that the major error in inhibitor – kinase binding energy calculations lies in the non-polar solvation terms of the solvation calculations. The quantum mechanically reverse calculated hydrophobic proxy factors for transfer from water to n-octane were 28.0 and 34.6 cal/(mol/Å²) for the imatinib ion and nilotinib respectively, which were very different from the arbitrary PBSA solvation values previously used in molecular mechanics literature studies. [4][Fong 2015]

Hydrophobicity or lipophilicity

As discussed above, the hydrophobic effect is a measure of the non-polar interaction between a drug and a protein within the binding pocket. Hydrophobicity is also related to lipophilicity, a concept widely used in the drug industry. The role of lipophilicity in drug discovery and design is a critical one. Lipophilicity is a key physicochemical property that plays a crucial role in determining the solubility and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties and the overall suitability of drug candidates. LogP, (the partition coefficient of a drug between water and n-octanol), is a widely used estimate of a compound's overall lipophilicity, a value that influence its behaviour in a range of biological processes relevant to a drug discovery, such as solubility, permeability through biological membranes, hepatic clearance, lack of selectivity and non-specific toxicity. For oral drugs, a logP value comprised between 2 and 3 is often considered optimal to achieve a compromise between permeability and first-pass clearance. [17,18,19][Hughes 2008, Arnott 2012,2013]

However, wet octanol solution is a poor proxy for proteins or biological membranes because it contains ~2M of water, and cyclohexane would be a much better proxy. A set of solvation parameters derived for the protein interior from protein engineering data was also different from octanol scale: it was close to cyclohexane scale for nonpolar atoms but intermediate between cyclohexane and octanol scales for polar atoms. [20,21][Radicza 1988, Lomize 2012]

Drug molecular volume Vs binding pocket volume

The molecular volume of a ligand can be easily measured, either as a bare molecule, or solvated. However the volume of a protein receptor pocket is more difficult to estimate, and the relationship between this volume and the volume of a ligand is unknown. An analysis of over 100 protein-ligand structures has shown that ligand volume and binding site volume are somewhat correlated when binding site volume is < or =700 Å³, but the ligand seldom occupies the entire site. [22][Liang 1998] A wide scale analysis of protein-ligand binding pockets reveals that the number of distinct pockets is small. Similar pockets occur in unrelated protein structures. The small number of pockets suggests that off-target interactions

among diverse proteins are inherent in kinases, proteases and phosphatases, and minor side effects cannot be avoided. For an example, sorafenib is one of FDA approved anti-cancer drugs that have multiple targets in the family of protein kinases. Two of these kinases, B-Raf and VEGFR2, have been crystallized in complex with the drug molecule and the structures show two structurally similar binding complex regions, despite a low global protein similarity. Protein kinases generally share high level structural similarity in its catalytic domain. [23][Skolnick 2015] These observations illustrate that within a certain protein pocket volume, and where the catalytic domain is similar, it may be possible to utilize the drug volume as one contributing key physical indicator to gauge drug-protein binding interaction. Similarly, where drug-active transporter interactions are involved, the drug molecular volume is also a critical factor. [2][Fong 2015] For diffusion controlled drug permeation and facilitated diffusion through cell membranes, it has been shown that molecular volume is a critical factor. [1][Fong 2014]

Quantitative structure activity models (QSAR) which seek to relate physico-chemical properties of drugs to biological binding or interaction with proteins or enzymes (eg K_m , K_i , IC_{50} etc) rarely have sufficient experimental data to be statistically robust, and the measurement accuracy of these properties varies very significantly from laboratory to laboratory. The measurement of physico-chemical properties of drugs are more accurate especially for small drugs less than about 500 Da, but errors can still be significant. To minimize errors in QSAR relationships, the use of comparative data from single sources is best, with rigorous statistical analysis, and the use of a minimal number of independent variables which have demonstrated independent physico-chemical rationales for their use.

In this study quantum mechanical studies of the TKI are applied, which have been conformationally optimised in water. Neutral and ionic species have been characterised based on the known pKa values of the drugs, and the four independent variables are calculated using the same high level computational method: comparative differences between these absolute calculations also minimize errors. This study will apply equation 1 to some competitive binding interactions of tyrosine kinase inhibition and to some competitive interactions of TKI with hOATP3, OATP, OCT influx transporters. [24,25,26][Kitagawa 2012, Minematsu 2011, Johnson 2014]

Chronic myeloid leukaemia (CML) is a cancer of blood-forming cells in the bone marrow. Abnormal cells gradually fill the bone marrow and spill into the bloodstream. The disease typically develops very slowly and symptoms such as anaemia, bleeding problems or infections may not occur for years after the disease starts. Bcr-Abl tyrosine-kinase inhibitors (TKI) are the first-line therapy for most patients with CML. Most cases of CML are caused by a chromosomal abnormality that results in the formation of a so-called Philadelphia chromosome which is a fusion between the Abelson (Abl) tyrosine kinase gene and the break point cluster (Bcr) gene, resulting in a chimeric oncogene (Bcr-Abl). This causes the production of tyrosine kinase. TKI inhibit the phosphorylation, a key step in the kinase activation of many proteins by signal transduction cascades. TKIs operate by four different mechanisms: they can compete with adenosine triphosphate (ATP), the phosphorylating agent, the substrate or both, or can act in an allosteric fashion, by binding to a site outside the active site, affecting its activity by a conformational change. [27][Posner 1994] Second-generation TKIs such as dasatinib and nilotinib have been approved for the treatment of CML patients who are refractory or intolerant to imatinib. In vitro tests of these new TKIs show considerably higher activity in comparison with imatinib, with a 40-fold increased potency for nilotinib and a 325-fold for dasatinib. Nilotinib and dasatinib are able to overcome

imatinib resistance caused by several BCR-ABL tyrosine kinase domain mutations. [28] [O'Hare 2005] A schematic representation of the interaction of a TKI with a tyrosine kinase is shown in Figure 1.

Results and discussion

Before absorption can occur a drug first has to be in a soluble state. The solubility of orally-administered drugs is dependent on their chemical properties and often by the intragastric pH the drug is exposed to in the process of absorption. Weakly basic drugs may show decreased absorption, while the absorption of weakly acidic drugs may increase at higher intragastric pH. Because kinase inhibitors (KI) are typically weakly basic, they can be present in either ionised or non-ionised forms. In vivo, the bioavailability of KI are pH dependent which depends upon the pKa of the KI as well as the pH of the gastrointestinal (GI) tract. For example, the solubility of imatinib and nilotinib rapidly declines above pH 5.5 and 4.5 respectively. Nilotinib or Tasigna/Nilotinib.HCl is practically insoluble in buffer solutions of pH 4.5 and higher pH values. (Novartis/ Tasigna fact sheet). Erlotinib becomes more soluble in a mildly acidic environment (pH 5.42) (Genentech fact sheet, 2013). Each segment of the GI tract has its own characteristic pH level: acidity declines over the GI tract from the stomach (pH 1-3) to the small intestine (pH 5-7) and the colon (pH 7-8). For imatinib and nilotinib solubility and absorption therefore rapidly decreases after the stomach. This is further supported by the relative short time to reach maximum plasma concentration (T_{max}) for these drugs; 2-4 hours for imatinib and 3 hours for nilotinib. Hence, due to the physicochemical properties of imatinib and nilotinib, the stomach is essential for dissolution and absorption of these TKIs. For sunitinib however, solubility does not decline until pH 6.8. Sunitinib is absorbed from the GI tract, reflected by a long time to reach maximum plasma concentration of sunitinib, e.g. 6-12 hours [29][de Wit 2014].

As many tyrosine-kinase inhibitors show pH-dependent solubility in the physiologically relevant pH range, they can be susceptible to gastric pH-dependent drug-drug interactions when co-administered with acid-suppressive agents. Such drug-drug interactions could reduce the systemic exposure of tyrosine kinase inhibitors, and lead to loss of therapeutic benefit. Concomitant use of acid-suppressing agents (eg proton pump inhibitors, H_2 antagonists, antacids) will shift the stomach environment to a higher pH, making it less acidic and, therefore, affecting the chemical bioavailability. [30][Guo 2014]

Imatinib, dasatinib, nilotinib, gefitinib, erlotinib, sorafenib, sunitinib, pazopanib, lapatinib are orally administered multi-targeted TKI with high activity towards several families of receptor and non-receptor tyrosine kinases involved in angiogenesis, tumour growth and metastatic progression of cancer. The absolute bioavailability in humans for imatinib is almost 100% with pazopanib between 14-39% and estimates have been made for nilotinib (31%), sorafenib (50%) and sunitinib (50%). The pH dependence of the solubility and concentrations of the ionized forms of drugs such as the TKI are critical factors in determining bioavailability. The ionized forms have a large bearing on the ability of drugs such as the TKI to be transported across cellular membranes, particularly where large free energies of desolvation are required before the TKI can bind with the enzyme in the binding pocket. [31][de Gion 2011, [4,5] [Fong 2014, 2015]

A significant issue relating to TKI (and other drugs) is what is the actual species involved in vivo compared to those used in vitro studies conducted during drug discovery and definition. Solubility and pKa are critical properties usually defined in vitro. There are laboratory

measurements and calculated values of pKa for most TKI, but few studies of what are the actual species involved in ionization processes. Using spectroscopic techniques, it has been shown that gefitinib protonates at the tertiary N of the morpholino group and at the N1 site of the quinazolino group, and lapatinib diprotonates at the secondary NH₂ and N1 of the quinazolino groups. [32][Song 2016] As several of the approved TKI have multiple basic sites, the actual species in vivo may have important clinical effects. A detailed study of the protonation sites of bosutinib shows 4 possible protonation sites, with experimental pKa values of 11.2 (aromatic amine), 8.1 (4-piperidine), 4.3 (quinoline) and 3.5 (1-piperidine). Noteworthy observations were the overlapping of the 8.1 and 4.3 values forming a diprotic system, and the potential diprotonation of the piperidine group. The latter observation may explain the X-ray crystal structures where imatinib (which contains the same piperidine group) binds to the kinase as the diprotonated piperidine species (3K5V, 3GVU). It is also notable that despite the pKa values indicating that only very small concentrations of the diprotonated species being present in solution, the diprotonated-kinase complex can still crystallise from solution. The use of computational techniques to examine multiple possible species that could exist in vitro and in vivo for both transport and binding, coupled with equation 1 can be useful diagnostic indicators in drug development.

The specificities of nine approved TKI (imatinib, dasatinib, nilotinib, gefitinib, erlotinib, lapatinib, sorafenib, sunitinib, and pazopanib) have been determined by activity-based kinase profiling using a large panel of human recombinant active kinases. Profiling was at the physiological level K_m and 1 mM for ATP to determine which kinases were susceptible to inhibition by the TKI. [24][Kitagawa [2013] The competitive inhibition (apparent K_m and IC_{50}) of ATP by various TKI have been analysed using equation 1 for the following kinases: ABL, DDR1, DDR2, PDGFR $_{\alpha}$, PDGFR $_{\beta}$ which have (minimally) sufficient data for analysis using equation 1.

The actual species in solution at pH 7 (the Kitagawa in vitro experimental conditions) have been determined from the known pKa values of the TKI (see Experimental). X-ray crystal structures have been examined where available to see if protonated inhibitors are bound to the kinase. Protonated forms of imatinib (2HYY, 3HEC PDB structures) and dasatinib (3D5G, 2GQG) have been found to bind to kinase enzymes, but there is no structural evidence that nilotinib (3CS9), erlotinib (1M17), sorafenib (3HEG), sunitinib (3GOE), pazopanib (3CJG), gefitinib (4WKQ), or lapatinib (1XKK) bind in the protonated forms. There are even examples where imatinib binds to the kinase as the diprotonated (on the piperidine moiety) species (3K5V, 3GVU). Sunitinib can bind in the protonated form (3MIY) and non-protonated form (3GOE) to different kinases. The complexities of thermodynamic control that govern complex crystallization versus kinetic control that govern determinations of K_m or IC_{50} under competitive binding conditions in pH dependent solubility conditions are difficult to disentangle. In many studies, protonated salts of the TKI are used to study in vitro binding to improve solubility of the inhibitor at pH 7. Using Kitagawa's pH 7 data, protonated forms of imatinib, dasatinib, gefitinib, sunitinib, the diprotonated form of lapatinib, and neutral forms of nilotinib, erlotinib, sorafenib and pazopanib were used, based on the form of the starting inhibitor as a salt or neutral base, pKa, and X-ray structures for the imatinib, dasatinib and sunitinib complexes. The same forms of species were used to examine Giacomini's in vitro hOCT3 transporter studies.

Kinase inhibition at a physiological ATP level of 1 mM ATP was used to profile of these tyrosine kinase inhibitors. K_m is the apparent value of ATP. The IC_{50} values for inhibitors against the various kinases studied are affected by the apparent K_m values. [24][Kitagawa

2012] Equation 2 and 3 show reasonable correlations for the TKI properties against K_m for the ABL tyrosine kinase. The $\Delta G_{\text{desolvation}}$ and $\Delta G_{\text{lipophilicity}}$ are calculated using the SMD solvation model and represent bulk solvation properties of the drug in water at pH 7. Eq 3 differs from eq 2 in using $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ where CDS is the cavitation dispersion solvent structure, involving non-bulk solvent electrostatic contributions to the free energy of hydration. The SMD solvation model is based on $\Delta G_S^{\circ} = \Delta G_{\text{ENP}} + G_{\text{CDS}}$ where ENP is the electronic nuclear polarization: the change in the solute free energy due to electrostatic interactions between the solute and the bulk solvent and distortion of the solute's electronic structure in solution. The solvent is modelled as a dielectric continuum. The CDS represents first solvation shell effects. It involves atomic surface tension (geometry dependent proportionality constants). The CDS has been parameterized using extensive experimental data sets for optimization, and has the advantage of including a realistic experimentally based hydrogen bonding model. The CDS covers *shorter-range* polarization effects and shorter-range non-electrostatic effects such as cavitation, dispersion, and solvent structural effects (which includes both hydrogen bonding) and exchange repulsion effects. [Marenich 2009] In this study it is postulated that the $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ values may be a close approximation of how a solvated inhibitor reacts as it approaches and starts to enter the kinase binding pocket (dielectric constant 20-30) and leaves the bulk water environment (dielectric constant 78.3) and desolvation binding starts to occur. This scenario has been modelled using more intensive molecular mechanics computations. [5-7][Setny 2010, Mondial 2014, Shan 2011 Eq 2 and 3 show similar sensitivities to the independent variables, but the signs are reversed for $\Delta G_{\text{desolvation}}$ and $\Delta G_{\text{desolv,CDS}}$. A positive term is expected for inhibitor desolvation as energy has to be expended to desolvate the incoming inhibitor, which is counter balanced by the hydrophobic ($\Delta G_{\text{lipophilicity}}$) and polar (dipole moment) interactions between the protein and inhibitor. Eq 3 shows a strong correlation between K_m for the TKI and the independent variables, showing the negative effect of desolvation and positive effect of the hydrophobic interaction (negative $\Delta G_{\text{lipo,CDS}}$) quite clearly.

Competitive inhibition of ATP by ABL tyrosine kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib, sorafinib, sunitinib ion, pazopanib **Equation 2**

$$K_m = -38.3\Delta G_{\text{desolvation}} -44.2\Delta G_{\text{lipophilicity}} +50.4 \text{ Dipole Moment} -20.7\text{Molecular Volume} + 6621.0$$

Where $R^2 = 0.939$, $SEE = 326.7$, $SE(\Delta G_{\text{desolvation}}) = 17.7$, $SE(\Delta G_{\text{lipophilicity}}) = 63.9$, $SE(\text{Dipole Moment}) = 44.0$, $SE(\text{Molecular Volume}) = 11.6$, $F=8.1$, $\text{Significance}=0.036$

Competitive inhibition of ATP by ABL tyrosine kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib, sorafinib, sunitinib ion, pazopanib where $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ are desolvation and lipophilicity free energies using the CDS solvation energy.

Equation 3

$$K_m = -76.9\Delta G_{\text{desolv,CDS}} -274.2\Delta G_{\text{lipo,CDS}} +20.5 \text{ Dipole Moment} -37.0\text{Molecular Volume} + 8398.4$$

Where $R^2 = 0.933$, $SEE = 343.0$, $SE(\Delta G_{\text{desolv,CDS}}) = 117.4$, $SE(\Delta G_{\text{lipo,CDS}}) = 121.1$, $SE(\text{Dipole Moment}) = 10.5$, $SE(\text{Molecular Volume}) = 6.7$, $F=10.4$, $\text{Significance}=0.041$

Most receptor tyrosine kinases are single subunit receptors, and ligand binding to the extracellular domain induces formation of receptor dimers. Each monomer has a single *hydrophobic* transmembrane-spanning domain, an extracellular N terminal region, and an

intracellular C terminal region. [33][Hubbard 1999]. Inhibition of receptor tyrosine kinases may be expected to a complex interplay involving these regions. Inhibition of several receptor tyrosine kinases have been analysed using equation 1: DDR1, DDR2, PDGFR α and PDGFR β .

DDR1 is a receptor tyrosine kinase involved in cancer cell invasion. Eq 4 and 5 show similar patterns to the equivalent eq 2 and 3 for the ABL kinase. Eq 5 shows a moderate correlation between K_m for the TKI and the independent variables, showing the negative effect of desolvation and positive effect of the hydrophobic effect (negative $\Delta G_{\text{lipo,CDS}}$) quite clearly. Eq 5 and 6 are similar showing that the comparison of K_m and IC_{50} seem to be valid, though the reliability of the individual coefficients is not high in view of the only moderate statistical correlations.

Competitive inhibition of ATP by DDR1 kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib, sorafinib, sunitinib ion, pazopanib, lapatinib diion

Equation 4

$$K_m = -11.5\Delta G_{\text{desolvation}} -97.7\Delta G_{\text{lipophilicity}} -78.5 \text{ Dipole Moment} +3.8\text{Molecular Volume} - 2102.2$$

Where $R^2 = 0.976$, $SEE = 315.8$, $SE(\Delta G_{\text{desolv}}) = 16.0$, $SE(\Delta G_{\text{lipo}}) = 34.0$, $SE(\text{Dipole Moment}) = 12.2$, $SE(\text{Molecular Volume}) = 3.8$, $F=40.8$, $\text{Significance}=0.002$

Competitive inhibition of ATP by DDR1 by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib, sorafinib, sunitinib ion, pazopanib, lapatinib diion where $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipoCDS}}$ are desolvation and lipophilicity free energies using the CDS solvation energy.

Equation 5

$$K_m = -418.7\Delta G_{\text{desolv,CDS}} -110.5\Delta G_{\text{lipo,CDS}} +6.8 \text{ Dipole Moment} +11.0\text{Molecular Volume} +8411.4$$

Where $R^2 = 0.727$, $SEE = 1068.1$, $SE(\Delta G_{\text{desolvCDS}}) = 117.4$, $SE(\Delta G_{\text{lipoCDS}}) = 374.2$, $SE(\text{Dipole Moment}) = 21.5$, $SE(\text{Molecular Volume}) = 20.2$, $F=2.7$, $\text{Significance}=0.180$

Competitive inhibition of ATP by DDR1 by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib, sorafinib, sunitinib ion, pazopanib, lapatinib diion where $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipoCDS}}$ are desolvation and lipophilicity free energies using the CDS solvation energy.

Equation 6

$$IC_{50} = -4.0\Delta G_{\text{desolv,CDS}} +22.5\Delta G_{\text{lipo,CDS}} +6.8 \text{ Dipole Moment} -1.5\text{Molecular Volume} +848.5$$

Where $R^2 = 0.775$, $SEE = 71.5$, $SE(\Delta G_{\text{desolvCDS}}) = 3.5$, $SE(\Delta G_{\text{lipoCDS}}) = 9.4$, $SE(\text{Dipole Moment}) = 21.5$, $SE(\text{Molecular Volume}) = 0.9$, $F=2.6$, $\text{Significance}=0.230$

Eq 7 and 8 for DDR2 appear to be quite different from the same equations for DDR1 even though they belong to the same family. This may be related to DDR1 and DDR2 being transmembrane receptors and the differences in receptor regions. Also erlotinib fits the equations best as the protonated form, based on the large outlier behaviour if treated as a neutral species. Eqn 7 and 8 are very similar, although only moderately correlated with K_m and IC_{50} .

Competitive inhibition of ATP by DDR2 tyrosine kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib ion, sorafinib, sunitinib ion, pazopanib

Equation 7

$$K_m = -20.0\Delta G_{\text{desolvation}} -91.0\Delta G_{\text{lipophilicity}} -44.9 \text{ Dipole Moment} -1.7\text{Molecular Volume} - 429.4$$

Where $R^2 = 0.877$, $SEE = 213.1$, $SE(\Delta G_{\text{desolvation}}) = 11.2$, $SE(\Delta G_{\text{lipophilicity}}) = 28.0$, $SE(\text{Dipole Moment}) = 11.7$, $SE(\text{Molecular Volume}) = 2.7$, $F=5.4$, $\text{Significance}=0.100$

Competitive inhibition of ATP by DDR2 kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib ion, sorafinib, sunitinib ion, pazopanib **Equation 8**

$$\mathbf{IC_{50} = -152.9\Delta G_{\text{desolvation}} - 715.0\Delta G_{\text{lipophilicity}} - 359.5 \text{ Dipole Moment} - 13.9\text{Molecular Volume} - 3372.5}$$

Where $R^2 = 0.893$, $SEE = 1574.3$, $SE(\Delta G_{\text{desolvation}}) = 82.6$, $SE(\Delta G_{\text{lipophilicity}}) = 205.0$, $SE(\text{Dipole Moment}) = 86.3$, $SE(\text{Molecular Volume}) = 20.1$, $F=3.8$, $\text{Significance}=0.150$

Eq 9-12 9-11 show moderate only correlations with PDGFR $_{\alpha}$ and PDGFR $_{\beta}$ kinases. Platelet-derived growth factor receptors (PDGFR) are cell surface tyrosine kinase receptors for members of the platelet-derived growth factor (PDGF) family. PDGF subunits α and β are important factors regulating cell proliferation, cellular differentiation, cell growth, development and many diseases including cancer. Also erlotinib fits the equations best as the protonated form, based on the large outlier behaviour if treated as a neutral species. Eq 9-12 show strong similarities with those for DDR2 but not DDR1.

Competitive inhibition of ATP by PDGFR $_{\alpha}$ kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib ion, sorafinib, sunitinib ion, pazopanib **Equation 9**

$$\mathbf{K_m = -19.4\Delta G_{\text{desolvation}} - 76.2\Delta G_{\text{lipophilicity}} - 31.0 \text{ Dipole Moment} - 2.5\text{Molecular Volume} - 45.6}$$

Where $R^2 = 0.902$, $SEE = 148.5$, $SE(\Delta G_{\text{desolvation}}) = 7.8$, $SE(\Delta G_{\text{lipophilicity}}) = 19.4$, $SE(\text{Dipole Moment}) = 8.2$, $SE(\text{Molecular Volume}) = 1.9$, $F=6.9$ $\text{Significance}=0.071$

Competitive inhibition of ATP by PDGFR $_{\alpha}$ kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib ion, sorafinib, sunitinib ion, pazopanib **Equation 10**

$$\mathbf{IC_{50} = -230.8\Delta G_{\text{desolvation}} - 921.5\Delta G_{\text{lipophilicity}} - 294.5 \text{ Dipole Moment} - 34.2\text{Molecular Volume} - 1520.6}$$

Where $R^2 = 0.918$, $SEE = 1474.4$, $SE(\Delta G_{\text{desolvation}}) = 77.6$, $SE(\Delta G_{\text{lipophilicity}}) = 194.0$, $SE(\text{Dipole Moment}) = 80.8$, $SE(\text{Molecular Volume}) = 18.8$, $F=8.4$, $\text{Significance}=0.055$

Competitive inhibition of ATP by PDGFR $_{\beta}$ kinase by imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib ion, sorafinib, sunitinib ion, pazopanib **Equation 11**

$$\mathbf{K_m = -23.7\Delta G_{\text{desolvation}} - 89.6\Delta G_{\text{lipophilicity}} - 36.7 \text{ Dipole Moment} - 2.1\text{Molecular Volume} - 259.2}$$

Where $R^2 = 0.898$, $SEE = 171.0$, $SE(\Delta G_{\text{desolvation}}) = 9.0$, $SE(\Delta G_{\text{lipophilicity}}) = 22.5$, $SE(\text{Dipole Moment}) = 9.4$, $SE(\text{Molecular Volume}) = 2.2$, $F=6.6$ $\text{Significance}=0.076$

Eq 12-15 show correlations of K_m and IC_{50} for TKI in inhibiting metformin uptake by the active transporter hOCT3. [26][Minematsu 2011] The organic cation uptake transporter 3 (OCT3) also known as the solute carrier family 22 member 3 (SLC22A3) is a protein that in humans is encoded by the *SLC22A3* gene. As the substrate concentration in the inhibition study was low compared with the Michaelis-Menten constant K_m , the IC_{50} values are virtually equal to the inhibition constant K_i values, assuming competitive or noncompetitive inhibition. Eq 13 correlation with $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ is similar to eq 12, but shows the negative effect of desolvation and the positive effect of the hydrophobic effect (ie negative $\Delta G_{\text{lipo,CDS}}$ lowers IC_{50}) quite clearly. Eq 14 show the correlation with IC_{50} assuming all the TKI are in the ionized form. Equation 15 shows the correlation assuming inhibitors are neutral species, which seems unlikely given the low solubilities at pH 7. However in view of the large desolvation penalties for the ionized forms, one possibility is the small concentrations of the neutral forms in equilibrium at pH 7 might be the preferred species transported by hOCT3. In vivo the situation is even more complex since it is known that TKI complex with blood serum and plasma proteins under clinical steady state conditions. TKI taken daily orally develop steady state plasma concentration within 2-3 weeks. [34][Honeywell]

Tyrosine kinase inhibitors (imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib, sorafenib, sunitinib ion, lapatinib diion) inhibiting active metformin uptake by hOCT3

Equation 12

$$IC_{50} = 0.13\Delta G_{\text{desolvation}} - 0.49\Delta G_{\text{lipophilicity}} - 0.75 \text{ Dipole Moment} - 0.07 \text{ Molecular Volume} - 11.5$$

Where $R^2 = 0.884$, $SEE = 6.91$, $SE(\Delta G_{\text{desolvation}}) = 0.35$, $SE(\Delta G_{\text{lipophilicity}}) = 0.75$, $SE(\text{Dipole Moment}) = 0.26$, $SE(\text{Molecular Volume}) = 0.08$, $F = 5.7$ $\text{Significance} = 0.090$

Tyrosine kinase inhibitors (imatinib ion, dasatinib ion, nilotinib, gefitinib ion, erlotinib, sorafenib, sunitinib ion, lapatinib diion) inhibiting active metformin uptake by hOCT3

Equation 13

$$IC_{50} = -8.7\Delta G_{\text{desolv,CDS}} - 3.4\Delta G_{\text{lipo,CDS}} + 0.3 \text{ Dipole Moment} - 0.1 \text{ Molecular Volume} - 77.2$$

Where $R^2 = 0.727$, $SEE = 1068.1$, $SE(\Delta G_{\text{desolvCDS}}) = 2.7$, $SE(\Delta G_{\text{lipoCDS}}) = 3.7$, $SE(\text{Dipole Moment}) = 3.0$, $SE(\text{Molecular Volume}) = 0.2$, $F = 2.5$, $\text{Significance} = 0.240$

Tyrosine kinase inhibitors (all ionic species) inhibiting active metformin uptake by hOCT3

Equation 14

$$IC_{50} = 0.83\Delta G_{\text{desolvation}} + 0.46\Delta G_{\text{lipophilicity}} + 0.11 \text{ Dipole Moment} - 0.26 \text{ Molecular Volume} + 205.0$$

Where $R^2 = 0.903$, $SEE = 7.83$, $SE(\Delta G_{\text{desolvation}}) = 0.55$, $SE(\Delta G_{\text{lipophilicity}}) = 0.98$, $SE(\text{Dipole Moment}) = 0.38$, $SE(\text{Molecular Volume}) = 0.16$, $F = 7.1$ $\text{Significance} = 0.070$

Tyrosine kinase inhibitors (all neutral species) inhibiting active metformin uptake by hOCT3

Equation 15

$$IC_{50} = 5.1\Delta G_{\text{desolvation}} - 3.8\Delta G_{\text{lipophilicity}} + 4.2 \text{ Dipole Moment} - 1.4 \text{ Molecular Volume} + 205.0$$

Where $R^2 = 0.953$, $SEE = 4.42$, $SE(\Delta G_{\text{desolvation}}) = 0.78$, $SE(\Delta G_{\text{lipophilicity}}) = 1.20$, $SE(\text{Dipole Moment}) = 0.91$, $SE(\text{Molecular Volume}) = 0.22$, $F = 15.1$ $\text{Significance} = 0.025$

Equations 16-18 Show the application of the general equation 1 to the organic anion transporting polypeptide OATP1A2, and the organic cation transporter OCT1 data of Johnson 2014 [26]. The inhibition of a wide range of multi-kinase inhibitors (MKI) to the uptake of the substrates anion E3S (estrone-3-sulphate) by OATP1A2 and the cation MPP⁺ (1-methyl-4-pyridium acetate) by OCT1 have been analysed. A critical question that arises in this study is the form of the inhibitor that interacts with anion and cation transporters, since the MKI are predominantly in the protonated form at pH 7, but small concentrations may exist in the neutral form as well, as discussed above in eq 12-15 above for the hOCT3 data.

Analysis of the raw IC_{50} data for OATP1A2 indicates that lapatinib, bosutinib and soratinib lie well outside the range of all other MKI, possibly indicating that these inhibitors are interacting in the protonated forms. There is also evidence (vide supra) that lapatinib and bosutinib can exist as diions. Statistical residual analysis suggests that lapatinib and bosutinib are better fits as diions, and soratinib is clearly an outlier (and can exist only as monoprotonated species). Eq 16 compares all MKI as ionic species with eq 17 with all MKI as neutral species (with lapatinib and bosutinib as diions in both eq 16 and 17).

Multi kinase inhibitors (gefitinib ion, lapatinib diion, nilotinib ion, sunitinib ion, bosutinib diion, vandetinib ion, afatinib ion, cediratinib ion, erlotinib ion, pelitinib ion, neratinib ion, foretinib ion) inhibiting active E3S uptake by OATP1A2

Equation 16

$$IC_{50} = -0.54\Delta G_{\text{desolvation}} - 2.21\Delta G_{\text{lipophilicity}} - 0.61 \text{ Dipole Moment} - 0.10 \text{ Molecular Volume} - 73.5$$

Where $R^2 = 0.957$, $SEE = 6.31$, $SE(\Delta G_{\text{desolvation}}) = 0.41$, $SE(\Delta G_{\text{lipophilicity}}) = 0.85$, $SE(\text{Dipole Moment}) = 0.20$, $SE(\text{Molecular Volume}) = 0.06$, $F=39.1$ Significance=0.000

Multi kinase inhibitors (gefitinib, lapatinib diion, nilotinib, sunitinib, bosutinib diion, vandetanib, afatinib, cediranib, erlotinib, pelitinib, neratinib, foretinib) inhibiting active E3S uptake by OATP1A2 **Equation 17**

$$IC_{50} = 0.16\Delta G_{\text{desolvation}} - 0.80\Delta G_{\text{lipophilicity}} - 0.71 \text{ Dipole Moment} - 0.02\text{Molecular Volume} - 5.3$$

Where $R^2 = 0.983$, $SEE = 4.00$, $SE(\Delta G_{\text{desolvation}}) = 0.28$, $SE(\Delta G_{\text{lipophilicity}}) = 0.58$, $SE(\text{Dipole Moment}) = 0.18$, $SE(\text{Molecular Volume}) = 0.02$, $F=101.3$ Significance=0.000

Eq 18 below compares with eq 16, but uses the $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ parameters. Multi kinase inhibitors (gefitinib ion, lapatinib diion, nilotinib ion, sunitinib ion, bosutinib diion, vandetanib ion, afatinib ion, cediranib ion, erlotinib ion, pelitinib ion, neratinib ion, foretinib ion) inhibiting active E3S uptake by OATP1A2 **Equation 18**

$$IC_{50} = -2.70\Delta G_{\text{desolv,CDS}} - 3.51\Delta G_{\text{lipo,CDS}} + 0.91 \text{ Dipole Moment} - 0.07\text{Molecular Volume} - 37.6$$

Where $R^2 = 0.821$, $SEE = 12.88$, $SE(\Delta G_{\text{desolv,CDS}}) = 2.80$, $SE(\Delta G_{\text{lipo,CDS}}) = 1.86$, $SE(\text{Dipole Moment}) = 0.27$, $SE(\text{Molecular Volume}) = 0.11$, $F=8.07$, Significance=0.009

Eq 19 below compares with eq 17, but uses the $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ parameters. Multi kinase inhibitors (gefitinib, lapatinib diion, nilotinib, sunitinib, bosutinib diion, vandetanib, afatinib, cediranib, erlotinib, pelitinib, neratinib, foretinib) inhibiting active E3S uptake by OATP1A2 **Equation 19**

$$IC_{50} = 4.21\Delta G_{\text{desolv,CDS}} - 2.83\Delta G_{\text{lipo,CDS}} - 0.07 \text{ Dipole Moment} + 0.40\text{Molecular Volume} - 118.6$$

Where $R^2 = 0.594$, $SEE = 19.45$, $SE(\Delta G_{\text{desolv,CDS}}) = 2.62$, $SE(\Delta G_{\text{lipo,CDS}}) = 3.7$, $SE(\text{Dipole Moment}) = 0.50$, $SE(\text{Molecular Volume}) = 0.20$, $F=2.56$, Significance=0.130

Equations 20-21 show the MKI inhibition of the uptake of the cation MPP^+ (1-methyl-4-pyridium acetate) by OCT1. The correlations are fairly poor quality, and include lapatinib and bosutinib as diions in both equations.

Multi kinase inhibitors (gefitinib ion, lapatinib diion, nilotinib ion, sunitinib ion, bosutinib diion, vandetanib ion, afatinib ion, cediranib ion, erlotinib ion, pelitinib ion, neratinib ion, foretinib ion, soratinib ion) inhibiting active MPP^+ uptake by OCT1 **Equation 20**

$$IC_{50} = -0.2\Delta G_{\text{desolvation}} - 0.15\Delta G_{\text{lipophilicity}} - 1.0 \text{ Dipole Moment} + 0.1\text{Molecular Volume} + 90.8$$

Where $R^2 = 0.400$, $SEE = 23.7$, $SE(\Delta G_{\text{desolvation}}) = 1.3$, $SE(\Delta G_{\text{lipophilicity}}) = 2.7$, $SE(\text{Dipole Moment}) = 0.7$, $SE(\text{Molecular Volume}) = 0.2$, $F=1.3$ Significance=0.345

Multi kinase inhibitors (gefitinib, lapatinib diion, nilotinib, sunitinib, bosutinib diion, vandetanib, afatinib, cediranib, erlotinib, pelitinib, neratinib, foretinib, soratinib ion) inhibiting active MPP^+ uptake by OCT1 **Equation 21**

$$IC_{50} = 0.18\Delta G_{\text{desolvation}} - 2.37\Delta G_{\text{lipophilicity}} - 2.60 \text{ Dipole Moment} - 0.01\text{Molecular Volume} - 5.3$$

Where $R^2 = 0.515$, $SEE = 21.2$, $SE(\Delta G_{\text{desolvation}}) = 1.46$, $SE(\Delta G_{\text{lipophilicity}}) = 3.28$, $SE(\text{Dipole Moment}) = 0.95$, $SE(\text{Molecular Volume}) = 0.12$, $F=2.11.3$ Significance=0.160

Eq 22 below compares with eq 20, but uses the $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ parameters. Multi kinase inhibitors (gefitinib ion, lapatinib diion, nilotinib ion, sunitinib ion, bosutinib diion, vandetanib ion, afatinib ion, cediranib ion, erlotinib ion, pelitinib ion, neratinib ion, foretinib ion, soratinib ion) inhibiting active MPP^+ uptake by OCT1 **Equation 22**

$$IC_{50} = -6.15\Delta G_{\text{desolv,CDS}} - 5.73\Delta G_{\text{lipo,CDS}} - 0.76 \text{ Dipole Moment} - 0.20\text{Molecular Volume} - 32.8$$

Where $R^2 = 0.668$, $SEE = 17.6$, $SE(\Delta G_{\text{desolv,CDS}}) = 4.06$, $SE(\Delta G_{\text{lipo,CDS}}) = 2.33$, $SE(\text{Dipole Moment}) = 0.44$, $SE(\text{Molecular Volume}) = 0.16$, $F=4.06$, $\text{Significance}=0.044$

Eq 23 below compares with eq 21, but uses the $\Delta G_{\text{desolv,CDS}}$ and $\Delta G_{\text{lipo,CDS}}$ parameters. Multi kinase inhibitors (gefitinib, lapatinib diion, nilotinib, sunitinib, bosutinib diion, vandetanib, afatinib, cediranib, erlotinib, pelitinib, neratinib, foretinib, soratinib ion) inhibiting active MPP^+ uptake by OCT1

Equation 23

$$\text{IC}_{50} = -10.04\Delta G_{\text{desolv,CDS}} - 6.68\Delta G_{\text{lipo,CDS}} - 1.02 \text{ Dipole Moment} - 0.29\text{Molecular Volume} - 31.0$$

Where $R^2 = 0.803$, $SEE = 13.54$, $SE(\Delta G_{\text{desolv,CDS}}) = 2.44$, $SE(\Delta G_{\text{lipo,CDS}}) = 1.95$, $SE(\text{Dipole Moment}) = 0.28$, $SE(\text{Molecular Volume}) = 0.10$, $F=8.16$, $\text{Significance}=0.006$

The above equations can only be indicative and not clearly definitive since competitive inhibition under the experimental conditions is clearly a complex series of reactions, and insufficient experimental data is not available to **derive** highly robust statistical correlations. However, a consistent pattern of correlations which emerges from the 23 equations all under different conditions, widely different TKI and MKI, various kinases and transporters gives confidence that the general equation 1 may have a widespread or even fairly universal application for small ligand-protein interactions.

Conclusions

It has been shown that equation 1 can be successfully applied to the competitive binding of a number of tyrosine kinase inhibitors and multi-kinase inhibitors to a range of kinase enzymes. Equation 1 also applies to the active competitive transport of these tyrosine kinase inhibitors by the hOCT3, OATP1A2 and OCT1 transporters. There is strong independent evidence from the literature that $\Delta G_{\text{desolvation}}$, $\Delta G_{\text{lipophilicity}}$, the dipole moment and molecular volume are good inherent indicators of the transport or binding ability of drugs.

Equation 1:

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

Or

$$\text{Transport or Binding} = \Delta G_{\text{desolv,CDS}} + \Delta G_{\text{lipo,CDS}} + \text{Dipole Moment} + \text{Molecular Volume}$$

A modified form of equation 1 using the free energy of water desolvation ($\Delta G_{\text{desolv,CDS}}$) and the lipophilicity free energy ($\Delta G_{\text{lipo,CDS}}$), where CDS represents the first solvation shell solvent properties, may be a good approximation of the drug approaching the entry of the protein receptor pocket or the surface of the protein transporter. Desolvation of water from the drug ($\Delta G_{\text{desolv,CDS}}$) before binding in the receptor pocket is required, and hydrophobic interactions between the drug and protein ($\Delta G_{\text{lipo,CDS}}$) is a positive contribution to binding.

Equation 1 or its modified form may be useful guides to drug discovery and design, particularly the allowing examination of the various species of a potential drug that may

predominate at different pH levels, or by making changes to the molecular structure to predict binding or transport properties.

Experimental

Computational methods

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. [35][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. [36,37][Rayne 2010, Rizzo 2006] 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 $SE(\Delta G_{\text{desolvation}})$, $SE(\Delta G_{\text{lipophilicity}})$, $SE(\text{Dipole Moment})$, $SE(\text{Molecular Volume})$, as calculated from “t” distribution statistics. Residual analysis was used to identify outliers.

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Inhibitors used by Kitagawa 2012 (from Table 3)

Imatinib mesylate, dasatinib, nilotinib, erlotinib hydrochloride, sorafenib tosylate, sunitinib malate, pazopanib, gefitinib, lapatinib ditosylate

Inhibitors used by Giacomini 2011 (from Supplementary Table S2)

Imatinib, dasatinib, nilotinib, gefitinib, erlotinib, sunitinib, lapatinib, sorafenib, gefitinib

Inhibitors used by Jones 2014 (from Table 1)

Gefitinib, lapatinib, nilotinib, sunitinib, bosutinib, vandetanib, afatinib, cediranib, erlotinib, pelitinib, neratinib, foretinib, soratinib

Solution properties of Inhibitors

Imatinib/Glivec base pKa (FDA) 7.7. **Glivec:** Imatinib methanesulfonate: pKa1 8.07; pKa2 3.73; pKa3 2.56; pKa4 1.52. ca 92% ionized at pH 7; Chemaxon major species protonated N4 piperidine group at pH 7.4

Dasatinib/Sprycel pKa basic 6.8, 3.1(pH saturated water solution 6.0) ca 39% ionized at pH 7. Chemaxon: major species protonated N4 piperidine group at pH 7

Nilotinib (FDA): basic 2.1, acid 5.4. ca 0% ionized at pH 7; Chemaxon: major species not protonated at pH 7; **Tasigna/Nilotinib.HCl** pKa basic 10.2, acidic 6.74 (ACD) ca 100% ionized at pH 7

Erlotinib.HCl/Tarceva pKa 5.42 (25C) ca 3% ionized at pH 7.0, mostly insoluble at pH 7, max solubility at pH 2. Aqueous solubility of erlotinib hydrochloride is dependent on pH, with increased solubility at a pH < 5 due to protonation of the secondary amine. Over the pH range of 1.4 to 9.6, maximal solubility of approximately 0.4 mg/mL occurs at a pH of approximately 2 (Tarceva: Roche fact sheet). Chemaxon: major species not protonated pH 7.4

Sorafenib pKa 2.0 basic, 11.55 acidic; Chemaxon: major species not protonated pH7.4; Sorafenib **Sorafenib Tosylate** pKa: 2.2 basic (ACD) (not ionized at pH 7)

Pazopanib/Votrient Pazopanib.HCl Chemaxon: major species not protonated pH 7; **free base:** pKa 6.4, 2.1 basic ionization sites, for pKa 6.4, ca 20% ionized at pH 7; It is very slightly soluble at pH 1 and practically insoluble above pH 4 in aqueous media. The pH of a 0.04% w/v solution of **pazopanib hydrochloride** in water is about 2.2

Sunitinib Maleate pKa 8.95 The solubility of sunitinib malate in aqueous media over the range pH 1.2 to pH 6.8 is in excess of 25 mg/mL. Chemaxon: major species protonated tert-N at pH 7.4. ca 99% ionized at pH 7

Gefitinib/Iressa pKa basic 5.42, 7.24. The drug substance exhibits basic properties, with two pKa values of 5.42 and 7.24, respectively. (Chemaxon pKa 6.9) The solubility of gefitinib in aqueous solution is pH dependent. At pH 3 it is sparingly soluble, while it is practically insoluble at pH 7. European Medicines Agency Evaluation of Medicines for Human Use At pH 7, gefitinib is ca 64% ionized. Protonated at tertiary N morpholino group.

Lapatinib Ditosylate: pKa basic 7.2, acid 16 (Chemaxon) ~ 61% protonated pH7, Doc.Ref.: EMEA/CHMP/563746/2008. Lapatinib pKa basic 7.2 Chemaxon: major species protonated at 2-(methylsulfonyl)ethyl]amino] group at pH 7.4; second protonation site quinazoline group ca pKa 5.4

Afatinib: basic pKa 8.8 Chemaxon: major species protonated $-\text{CH}_2\text{N}^+(\text{H})\text{Me}_2$ at pH 7, 98% protonated at pH 7

Bosutinib: basic pKa 8.0, 4.8, which can overlap to form monoprotic and diprotic species at ca pH 7.

Box KJ, Donkor RE, Jupp PA, Leader IP, Trew DF, Turner CH. The chemistry of multi-protic drugs Part 1: a potentiometric, multi-wavelength UV and NMR pH titrimetric study of the micro-speciation of SKI-606. J. Pharm. Biomed. Anal. 2008, 47, 303-11.

Cediranib: Chemaxon: Major species protonated at $\text{N}^+(\text{H})$ pyrrolidine at pH 7

Foretinib: basic pKa 7.0 ACD, 50% protonated at pH 7

Neratinib: basic pKa 8.0 ACD, Chemaxon: major species protonated $-\text{CH}_2\text{N}^+(\text{H})\text{Me}_2$ at pH 7, 91% protonated at pH 7

Pelitinib: basic pKa 8.0, 12.2, Chemaxon: Major species protonated $-\text{CH}_2\text{N}^+(\text{H})\text{Me}_2$ at pH 7, 91% protonated at pH 7

Vandetanib: basic pKa 9.4, Chemaxon: Major species protonated N4 piperidine group at pH 7, 99% protonated at pH 7

X-ray structures of inhibitor-tyrosine kinase complexes from PDB data base indicating whether inhibitor is protonated in complex

2HY Y: Cowan-Jacob, S.W., Fendrich, G., Floersheimer, A., Furet, P., Liebetanz, J., Rummel, G., Rheinberger, P., Centeleghe, M., Fabbro, D., Manley, P.W. Structural biology contributions to the discovery of drugs to treat chronic myelogenous leukaemia. (2007) Acta Crystallogr., Sect. D 63: 80-93 **Imatinib:** N-protonated piperidine ring

3K5V: Zhang, J., Adrian, F.J., Jahnke, W., Cowan-Jacob, S.W., Li, A.G., Iacob, R.E., Sim, T., Powers, J., Dierks, C., Sun, F., Guo, G.R., Ding, Q., Okram, B., Choi, Y., Wojciechowski, A., Deng, X., Liu, G., Fendrich, G., Strauss, A., Vajpai, N., Grzesiek, S., Tuntland, T., Liu, Y., Bursulaya, B., Azam, M., Manley, P.W., Engen, J.R., Daley, G.Q., Warmuth, M., Gray, N.S. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. (2010) Nature 463: 501-506 **Imatinib:** protonated at both piperidine N sites

3GVU: Salah, E., Ugochukwu, E., Barr, A., Mahajan, P., Shrestha, B., Savitsky, P., Knapp, S. The crystal structure of human ABL2 in complex with GLEEVEC **Imatinib:** protonated at both piperidine N sites

3CS9: Weisberg, E., Manley, P.W., Breitenstein, W., Brueggen, J., Cowan-Jacob, S.W., Ray, A., Huntly, B., Fabbro, D., Fendrich, G., Hall-Meyers, E., Kung, A.L., Mestan, J., Daley, G.Q., Callahan, L., Catley, L., Cavazza, C., Azam, M., Neuberg, D., Wright, R.D., Gilliland, D.G., Griffin, J.D. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl, (2005) Cancer Cell 7: 129-141 **Nilotinib:** not protonated

2GQG: Tokarski, J.S., Newitt, J., Chang, C.Y.J., Cheng, J.D., Wittekind, M., Kiefer, S.E., Kish, K., Lee, F.Y.F., Borzilleri, R., Lombardo, L.J., Xie, D., Zhang, Y., Klei, H.E. The Structure of Dasatinib (BMS-354825) Bound to Activated ABL Kinase Domain Elucidates Its Inhibitory Activity against Imatinib-Resistant ABL Mutants, (2006) Cancer Res. 66: 5790-5797 **Dasatinib:** protonated at piperidine at N₆-(CH₂)₂OH site

3G5D: Getlik, M., Grutter, C., Simard, J.R., Kluter, S., Rabiller, M., Rode, H.B., Robubi, A., Rauh, D. Hybrid compound design to overcome the gatekeeper T338M mutation in cSrc, (2009) J. Med. Chem. 52: 3915-3926 **Dasatinib:** protonated piperidine ring at N₆-(CH₂)₂OH site,

1M17: Stamos, J., Sliwkowski, M.X., Eigenbrot, C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. (2002) J. Biol. Chem. 277: 46265-4627 **Gefitinib:** not protonated

4WKQ: Yosaatmadja, Y., Squire, C.J., McKeage, M., Flanagan, J.U. 1.85 angstrom structure of EGFR kinase domain with gefitinib shows zwitterionic 2-(N-morpholino)-ethanesulfonic Acid interaction with Lys879A. **Gefitinib:** not protonated.

3HEC, 3HEG: Namboodiri, H.V., Bukhtiyarova, M., Ramcharan, J., Karpusas, M., Lee, Y., Springman, E.B. Analysis of imatinib and sorafenib binding to p38alpha compared with

c-Abl and b-Raf provides structural insights for understanding the selectivity of inhibitors targeting the DFG-out form of protein kinases. (2010) *Biochemistry* 49: 3611-3618

Sorafenib-p38alpha kinase compared to imatinib-c-ABL Xray structure in co-crystals of two complexes: 3HEG for sorafenib-p38alpha, **Sorafenib**: no protonated piperidine ring sites. 3HEC for **imatinib**-p38alpha, shows 2 protonated piperidine ring sites. A shared feature of these drugs is the fact that they both bind to the DFG-out forms of their kinase targets.

1XKK: E.R.Wood et al. (2004). A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res*, 64, 6652-6659. **Lapatinib**-EGFR kinase Xray: no protonated sites on lapatinib

3MIY: Kutach, A.K., Villasenor, A.G., Lam, D., Belunis, C., Janson, C., Lok, S., Hong, L.N., Liu, C.M., Deval, J., Novak, T.J., Barnett, J.W., Chu, W., Shaw, D., Kuglstatter, A. Crystal structures of IL-2-inducible T cell kinase complexed with inhibitors: insights into rational drug design and activity regulation. (2010) *Chem.Biol.Drug Des.* 76: 154-163. **Sunitinib**: protonated at N(H⁺)Et₂ site

3GOE: Gajiwala, K.S., Wu, J.C., Christensen, J., Deshmukh, G.D., Diehl, W., DiNitto, J.P., English, J.M., Greig, M.J., He, Y.A., Jacques, S.L., Lunney, E.A., McTigue, M., Molina, D., Quenzer, T., Wells, P.A., Yu, X., Zhang, Y., Zou, A., Emmett, M.R., Marshall, A.G., Zhang, H.M., Demetri, G.D. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and **sunitinib** in gastrointestinal stromal tumor patients. (2009) *Proc.Natl.Acad.Sci.USA* 106: 1542-1547 **Sunitinib**: Not protonated at NEt₂ group

3CJG: Harris, P.A., Bloor, A., Cheung, M., Kumar, R., Crosby, R.M., Davis-Ward, R.G., Epperly, A.H., Hinkle, K.W., Hunter, R.N., Johnson, J.H., Knick, V.B., Laudeman, C.P., Luttrell, D.K., Mook, R.A., Nolte, R.T., Rudolph, S.K., Szewczyk, J.R., Truesdale, A.T., Veal, J.M., Wang, L., Stafford, J.A. Discovery of 5-[[4-[(2,3-dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methyl-benzenesulfonamide (**Pazopanib**), a novel and potent vascular endothelial growth factor receptor inhibitor. (2008) *J.Med.Chem.* **51**: 4632-4640. **Pazopanib**: Not protonated

1M17: Stamos, J., Sliwkowski, M.X., Eigenbrot, C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. (2002) *J.Biol.Chem.* 277: 46265-46272 **Erlotinib**: not protonated

3UE4: Levinson, N.M., Boxer, S.G. Structural and spectroscopic analysis of the kinase inhibitor bosutinib and an isomer of bosutinib binding to the abl tyrosine kinase domain. (2012) *Plos One* 7: e29828-e29828 **Bosutinib**: diprotonated at piperidine group

4G5J: Solca, F., Dahl, G., Zoepfel, A., Bader, G., Sanderson, M., Klein, C., Kraemer, O., Himmelsbach, F., Haaksma, E., Adolf, G.R. Target Binding Properties and Cellular Activity of Afatinib (BIBW 2992), an Irreversible ErbB Family Blocker. (2012) *J.Pharmacol.Exp.Ther.* **343**: 342-350 **Afatinib**: protonated at -CH₂N⁺(H)Me₂ group

2JIV: Yun, C.-H., Mengwasser, K.E., Toms, A.V., Woo, M.S., Greulich, H., Wong, K.-K., Meyerson, M., Eck, M.J. The T790M Mutation in Egfr Kinase Causes Drug Resistance

by Increasing the Affinity for ATP. (2008) Proc.Natl.Acad.Sci.USA **105**: 2070 **Neratinib**: protonated at $-\text{CH}_2\text{N}^+(\text{H})\text{Me}_2$ group

3LQ8: Qian F, Engst S, Yamaguchi K, Yu P, Won KA, Mock L, Lou T, Tan J, Li C, Tam D, Loughed J, Yakes FM, Bentzien F, Xu W, Zaks T, Wooster R, Greshock J, Joly AH Inhibition of tumor cell growth, invasion, and metastasis by EXEL-2880 (XL880, GSK1363089), a novel inhibitor of HGF and VEGF receptor tyrosine kinases. *Cancer Res.* **69** 8009-16 (2009) **Foretinib**: not protonated

2IVU: Knowles, P.P., Murray-Rust, J., Kjaer, S., Scott, R.P., Hanrahan, S., Santoro, M., Ibanez, C.F., McDonald, N.Q. Structure and Chemical Inhibition of the Ret Tyrosine Kinase Domain. (2006) *J.Biol.Chem.* **281**: 33577 **Vandetanib**: no information on protonation status of ligand

Table 1 Calculated molecular parameters for tyrosine kinase inhibitors

Tyrosine Kinase Inhibitor	$\Delta G_{\text{desolvation}}$ kcal/mol (CDS) Water	$\Delta G_{\text{lipophilicity}}$ kcal/mol (CDS) n-Octane	Dipole Moment D Water	Molecular Volume cm^3/mol Water
Imatinib	27.5 (3.0)	-24.7 (-15.6)	7.1	339
Dasatinib	34.6 (3.2)	-24.4 (-13.9)	4.2	359
Nilotinib	25.0 (8.8)	-24.8 (-14.1)	13.1	356
Erlotinib	25.0 (9.7)	-18.7 (-9.9)	9.0	323
Sorafenib	21.6 (10.3)	-17.8 (-9.3)	3.3	280
Sunitinib	23.0 (7.5)	-16.8 (-8.7)	5.4	294
Pazopanib	28.9 (6.0)	-22.5 (-11.5)	15.7	310
Gefitinib	21.5 (5.5)	-18.9 (-11.2)	10.6	312
Lapatinib	39.8 (8.2)	-28.8 (-14.9)	10.2	383
Bosutinib	78.0 (12.7)	-45.6 (-8.8)	50.2	423
Vandetanib	15.3 (3.2)	-17.7 (-11.9)	6.1	281
Afatinib	35.2 (5.8)	-23.7 (-12.5)	6.7	333
Cediranib	18.6 (5.8)	-18.5 (-10.8)	2	292
Pelitinib	25.4 (7.8)	-17.7 (-7.5)	6.4	309
Neritinib	32.0 (8.0)	-22.6 (-10.6)	4.7	388
Foretinib	33.8 (11.0)	-22.8 (-11.2)	8.8	468
InatinibH Ion	82.0 (7.2)	-52.7 (-15.8)	46.0	377
DasatinibH Ion	95.8 (6.8)	-53.4 (-14.1)	41.1	371
NilotinibH Ion	77.9 (11.0)	-51.6 (-14.5)	50.3	338
ErlotinibH Ion	72.0 (10.8)	-39.9 (-9.4)	10.4	324
SorafenibH Ion	74.7 (11.3)	-43.0 (-9.4)	33.9	314
SunitinibH Ion	72.5 (10.5)	-42.0 (-8.8)	26.0	320
PazopanibH Ion	77.5 (6.9)	-45.2 (-11.5)	24.4	314
GefitinibH Ion	59.8 (7.8)	-47.7 (-11.3)	36.7	282
LapatinibHH Diion	188.9 (11.9)	-100.5 (-15.4)	36.5	437
LapatinibH Ion	106.1 (10.1)	-58.4 (-15.1)	35.2	373
BosutinibH Ion	78.0 (12.7)	-45.6 (-8.8)	73.2	465

BosutinibHH Diion	216.1 (12.7)	-113.1 (-8.8)	73.2	465
VandetanibH Ion	71.3 (7.3)	-46.3 (-12.1)	44.9	335
AfatinibH Ion	94.7 (10.2)	-53.5 (-12.8)	26.4	332
CediranibH Ion	75.8 (8.6)	-46.4 (-11.0)	32.1	337
PelitinibH Ion	81.5 (12.1)	-46.4 (-7.7)	33.3	408
NeritinibH Ion	89.0 (12.3)	-51.3 (-10.8)	34.5	361
ForetinibH Ion	82.1 (14.3)	-47.7 (-11.4)	41.0	449

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