JOURNAL OF CHEMICAL INFORMATION AND MODELING

Article

pubs.acs.org/jcim

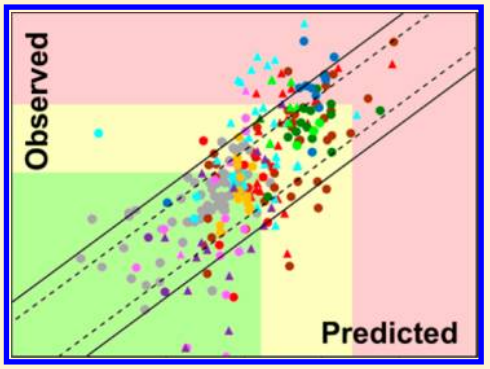
In silico Prediction of Total Human Plasma Clearance

Giuliano Berellini,[†] Nigel J. Waters,[†,‡] and Franco Lombardo*[,†]

[†]Metabolism and Pharmacokinetics, Novartis Institutes for Biomedical Research, 250 Massachusetts Avenue, Cambridge Massachusettes 02139, United States

Supporting Information

ABSTRACT: The prediction of the total human plasma clearance of novel chemical entities continues to be of paramount importance in drug design and optimization, because it impacts both dose size and dose regimen. Although many in vivo and in vitro methods have been proposed, a well-constructed, well-validated, and less resource-intensive computational tool would still be very useful in an iterative compound design cycle. A new completely in silico linear PLS (partial least-squares) model to predict the human plasma clearance was built on the basis of a large data set of 754 compounds using physicochemical descriptors and structural fragments, the latter able to better represent biotransformation processes. The model has been validated using the "ELASTICO" approach (Enhanced Leave Analog-Structural, Therapeutic, Ionization Class Out) based on ten therapeutic/structural analog classes. The model yields a geometric mean fold error (GMFE) of 2.1 and a percentage of compounds predicted within 2- and 3-fold error of 59% and 80%, respectively, showing an improved performance when compared with previous published works in predicting clearance of neutral compounds, and a very good performance with ionized molecules at pH 7.5, able to compare favorably with fairly accurate in vivo methods.



INTRODUCTION

Clearance is a very important pharmacokinetics (PK) parameter because it influences both half-life (together with the volume of distribution) and bioavailability (together with oral absorption) and thus impacts dose regimen (*how often*) and dose size (*how much*) of a drug. Therefore, the prediction of the total human plasma clearance of novel chemical entities is highly sought after in drug design and optimization. Its prediction helps determine the feasibility of clinical dosing and provides a framework for starting dose for first in human studies, for an estimation of clinical efficacious dose when coupled with pharmacokinetics/pharmacodynamics (PD) understanding of drug candidates.

Many in vivo methods have been proposed to predict human clearance, and they have traditionally been based on single or multiple in vivo preclinical species mainly rat, dog, and monkey.[1−13] These methods, however, are generally resource-intensive, time-consuming, and coupled with significant cost with a prediction accuracy nearing 60−65% of compounds within a 2-fold error, the latter being a seemingly accepted threshold in the field. This was shown, in particular, by a recent report where in vivo methods have been compared on the basis of a large set of experimental human and animal PK data.[14] These authors show the difficulties in predicting the human clearance within 2-fold of accuracy, and the comparison highlights the need of nonrodent species, in particular monkey, the latter becoming of crucial importance for neutral compounds.

In vitro methods have of course been proposed based on microsomal or hepatocyte data. However, while they are less expensive when compared to in vivo studies, they are generally limited to the prediction of a particular elimination pathway and often have to rely on the assumption that the total clearance is mainly hepatic, renal, and/or biliary.[15−25] Furthermore, to achieve some confidence in their application, some in vivo data are necessary to show that the scaling of clearance based on in vitro data is in fact comparable with observed in vivo clearance, that is, an in vitro−in vivo correlation can be established.

The reliance on a sizable number of in vitro and in vivo approaches, and the lack of reliability of any single one in different circumstances and with different compounds, might be taken as an indication of the difficulty encountered in predicting human clearance, and it may also explain the paucity of successful in silico models.[26−28] Yap et al.[26] presented models based on mixed physicochemical and topological descriptors, relying on several statistical approaches. Yu[27] reported a *k*NN (*k*-nearest-neighbors) model based on the similarity of compounds using common physicochemical descriptors, but applicable, with a certain accuracy, only to the ionized compounds. More recently, Demir-Kavuk et al.[28] presented an application to predict clearance based on molecular descriptors and topological fingerprints taken from several software packages. It combines feature generation and feature selection to reduce the number of descriptors, model building, and control of overtraining by using quadratic and linear regularization terms.

Received: March 22, 2012

   dx.doi.org/10.1021/ci300155y | *J. Chem. Inf. Model.* XXXX, XXX, XXX−XXX

However, a well-constructed, well-validated computational tool would still be very useful in a drug discovery setting, allowing proposed molecules to be tested early and prior to synthesis and screening. Here we describe and compare, against the better performing in vivo methods, a novel computational model, based on the combined use of physicochemical descriptors and structural fragments, modeled using a linear PLS[29] (partial least-squares) regression. The result is a completely in silico interpretative model, able to predict the total human plasma clearance based on a fairly large set of compounds.

RESULTS AND DISCUSSION

As a first exploratory approach, several linear partial least-squares (PLS[29]) models were built to correlate the logarithm of human clearance with only physicochemical descriptors, taking various starting points in terms of descriptors and principal components (PC). As a second step, we applied other statistical tools such as random forest[30] (RF), rule-based,[31] or models based on the compound similarity as described in the Methods section, to try to increase the accuracy of prediction by using nonlinear approaches. The latter methods did not show any significant improvement (data not shown) and a PLS model, showing the highest performance, yielded a total "leave-one-out" (LOO) geometric mean fold error (GMFE) of 2.5 and a GMFE of 3.1 and 2.3 for neutral and ionized compounds, respectively. These results, although comparable to the previous reported in silico models,[27,28] are still inadequate, and they prompted us toward a new approach based on the addition of structural fragments able to better represent the biotransformation processes than would the use of physicochemical descriptors alone.

The addition of structural fragments to the physicochemical descriptors did show in general an improvement for all models with all statistical tools used. The best PLS model turned out to be based on only 63 physicochemical descriptors and structural fragments, selected as explained in the Methods section, and it yielded a "leave-one-out" (LOO) GMFE of 2.2 and a percentage of compounds predicted within 2- and 3-fold of the actual value of 55% and 75%, respectively, with a maximum fold error of 99. The RF models, either based on 63 final selected physicochemical descriptors and structural fragments, or on all the approximately 1500 original fragments along with physicochemical descriptors, yielded a best out-of-bag (OOB) GMFE of 2.3 and an accuracy of prediction within 2- and 3-fold of 53% and 73%, respectively, with a maximum fold error of 210.

An analysis of performance along ionization classes, for RF models yielded an OOB GMFE of 2.9 and 2.1 for neutral and ionized compounds at pH 7.5, respectively. In particular the RF models showed a better GMFE of 2.4, 2.1, and 1.7 for anionic, cationic, and zwitterionic compounds, respectively. Neutral compounds yielded a PLS LOO GMFE of 2.6 while compounds ionized at pH 7.5 yielded a PLS LOO GMFE of 2.1, on the basis of values of 2.3 for anionic, 2.1 for cationic, and 1.6 for zwitterionic compounds. These results demonstrate that the linear PLS models have a similar or higher performance when compared with other statistical tools described as nonlinear. Therefore, in our opinion, PLS would represent a better choice due to its higher level of interpretability.

In fact the described PLS model showed a general improvement in prediction accuracy compared with previous published work and largely based on the same data.[27,28] Yap did not report the performance of their in silico model according to the ionization state of compounds, and their model has only been validated using part of the data as an external test set.[26] Although based on a different collection of data sets, grouped in a commercial database, not reporting the references of each original human study, a comparison based on the compounds present in our data set and in their external data set was performed. It is interesting to note, however, that the 73 compounds of the Yap test set that are in common with ours show very similar human values, as it might be expected, but not the same for all. Values of 0.8 for r^2 and GMFE of 1.3 were observed when the two sets of 73 compounds were compared plotting the values of clearance from the different sets against each other. This is indicative of data collection differences, always lurking when complex and variable end-points such as human clearance are considered, which may be traced, for example, across cohorts of subjects, to weight-averaged values vs values at a single dose, area-under-the-curve based on total radioactivity rather than on parent compounds, nonlinear pharmacokinetics, sometimes even including oral studies and other possible differences. Also, when this set of 73 compounds was used as an external test set, our PLS model yielded a GMFE of 1.7, matching the best performance reported by Yap et al., and the percentages of accurate predictions within 2- and 3-fold were 64% and 92% based on our human data values and 74% and 93% when the human data values reported by Yap et al. were considered. We note that when a model shows a significantly higher performance with an external rather with a total data set, that may suggest a bias of testing compounds and they may be not representative of all compounds.

Although a "leave-one-out" (LOO) approach could give a first estimation of goodness of a model, we are of the opinion that a leave-class-out approach as well as an analysis of the performance of ionization classes is a far more rugged test. We termed our approach with the acronym ELASTICO (Enhanced Leave Analog Structural Therapeutic Ionization Class Out) which is an extension of leave-class-out (LCO) of therapeutic analogs already used in previous works to validate models[32−34] and it is described in the Methods section. We believe it represents a more robust and realistic cross-validation method compared to the "leave-one-out" (LOO) approach or to approaches considering only the ionization classes, especially in a discovery setting, when the model is expected to be applied to novel series of analogs. The 261 compounds that form the basis of the ELASTICO validation test set represent quite well the total chemical space of all 754 compounds as shown in Figure 1.

In Table 1 we report the statistical results of in silico PLS model according to the ELASTICO validation. Across all 261 compounds, the PLS model shows a very good prediction with an overall GMFE of 2.1 and an accuracy of prediction within 2- and 3-fold of 59% and 80%, respectively. When the results according to the ionization classes are taken into account, there is a strong improvement in the prediction of clearance for ionized compounds, in particular cationic and zwitterionic, but we observed the same difficulties encountered in predicting neutral compounds by other authors.[27] In fact, as shown in Figure 2, the model is able to predict with an accuracy around 2-fold almost all classes, ionized compounds and in particular zwitterionic compounds such as β-lactams and fluoroquinolones, but also cationic compounds such as tri- and tetra-cyclic antidepressants, α- and β-adrenergic agonists and antagonists, and, partially, the morphinans. However, it is less accurate with

dx.doi.org/10.1021/ci300155y | J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

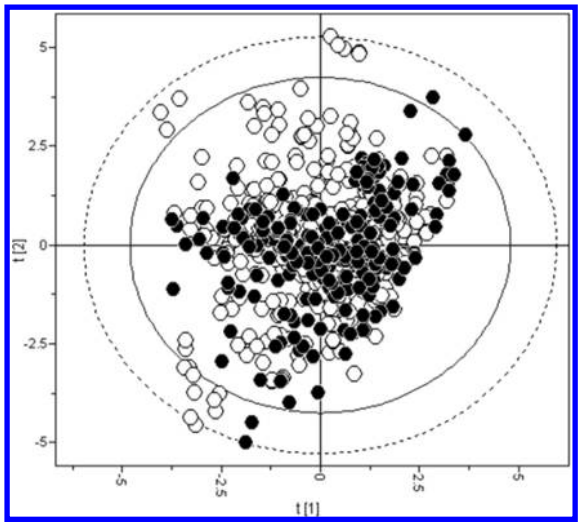


Figure 1. PLS score plot (PC 2 vs PC 1) of the model generated on all 754 compounds (unfilled circles) using the 63 descriptors showing the distribution of 261 compounds of ELASTICO test set (filled circles). Only the compounds inside the *t*-Hotelling plot at 95% (internal oval) and 99% (external oval, dotted line) degree of confidence are shown for clarity of representation.

anionic compounds, and in particular the NSAIDs, with a GMFE greater than 4, which is a class that moreover encompasses a range of values spanning almost 3 orders of magnitude (0.03 of tenoxicam up to 21 mL/min/kg of phenacetin). On the other hand, and with the exclusion of steroids, all classes mainly populated by neutral compounds such as nucleosides and benzodiazepines show a lower accuracy in prediction. A qualitative classification analysis has also been performed on the ELASTICO validation test set, by partitioning the compounds in three bins according to thresholds of *low* (when less than 4 mL/min/kg, <20% human hepatic blood flow (HBF)), *medium* (between 4 and 16 mL/min/kg, 20−80% HBF) and *high* (greater than 16 mL/min/kg, >80% HBF) clearance. The percentage of well-classified compounds according to three bins for the ELASTICO validation test set is reported in Table 1 and also presented in Figure 2, with an overall 65% of compounds well classified. Of course, all classes showing a good GMFE, also show a good binning capacity such as β-lactams and tri and tetracyclic antidepressants, but it is very significant that the NSAIDs and benzodiazepines are qualitatively well-predicted as low clearance with a 76% and 72% of compounds correctly binned, respectively, although their poor quantitative prediction with a GMFE of 4.3 and 2.6 with a 35% and 33% of compounds predicted within 2-fold, and a 53% and 72% of compounds predicted within 3-fold, respectively. That is, when the clearance is very low, the quantitative prediction tends to overestimate the observed clearance in terms of fold error, but it is still qualitatively quite good. However, while the low and high bins may offer a clear trend, the 4-fold range of the middle bin is wide and does not allow even a "best" or "worst" case estimation. Therefore the in silico PLS model is able to qualitatively identify *low-clearance* compounds as shown in Figure 2, where almost all compounds with low clearance are inside the predicted green region although far from the best fit. On the contrary, the model tends to underestimate the compounds with higher clearance such as morphinans and nucleosides/nucleotides showing a poor quantitative (2.5 and 2.8 of GMFE) and qualitative prediction (33% and 51% of corrected binned compounds, respectively). It is important to note that the model never bins low or medium clearance compounds as high, therefore, when the in silico model predicts a compound to have a high clearance, there is a high level of confidence that it will be so in human.

Table 1. ELASTICO Validation of in silico PLS Model

class	#	Observed CL average (range) (mL/min/kg)	GMFE	% < 2-fold	% < 3-fold	max fold error	% bin confidence
β-lactams (penams, penems, ...)	33	3.4 (0.4 - 7.6)	1.6	70	85	5	
β-lactams (cephalosporines)	34	2 (0.3 - 3.9)	1.5	82	97	5	
all β-lactams	67	2.7 (0.3 - 7.6)	**1.6**	**76**	**93**	5	**84**
β adrenergic (agonists & antagonists)	30[a]	21.9 (2 - 290)	2.1	57	87	33	
α adrenergic (agonists & antagonists)	17[a]	4.4 (0.5 - 11)	1.9	65	88	10	
all α, β adrenergic	44[a]	15.6 (0.5 - 290)	**2.1**	**57**	**87**	33	**57**
tri- & tetra-cyclic antidepressants	14	11.5 (2.5 - 21.9)	**1.6**	**86**	**100**	3	**71**
steroids	29	9.1 (0.3 - 36)	**2.2**	**52**	**72**	8	**45**
nucleosides & nucleotides	35	16.3 (0.9 - 130)	**2.8**	**46**	**60**	26	**51**
NSAIDs	17	3.4 (0.03 - 21)	**4.3**	**35**	**53**	98	**76**
morphinans	12	23 (2.2 - 57)	**2.5**	**25**	**75**	7	**33**
fluoroquinolone antibiotics	15	3.2 (1.2 - 8.3)	**1.5**	**93**	**100**	3	**73**
Ca blockers (dihydropyridine)	10	26.9 (7 - 142)	**2.5**	**50**	**70**	28	**70**
benzodiazepines	18	2.5 (0.1 - 16)	**2.6**	**33**	**72**	15	**72**
neutral	79	13.8 (0.1 - 142)	**2.7**	**42**	**65**	28	
ionized	182	8.1 (0.03 - 290)	**1.9**	**66**	**87**	98	
anionic	71	2.7 (0.03 - 12)	**2.2**	**58**	**79**	98	
cationic	73	16 (0.5 - 290)	**2.0**	**62**	**88**	33	
zwitterionic	38	2.9 (0.4 - 8.3)	**1.4**	**89**	**100**	3	
all	261	9.9 (0.03 - 290)	**2.1**	**59**	**80**	98	**65**

[a]Carvedilol, Etilefrine, and Labetalol are both α- and β-adrenergic.

dx.doi.org/10.1021/ci300155y | J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

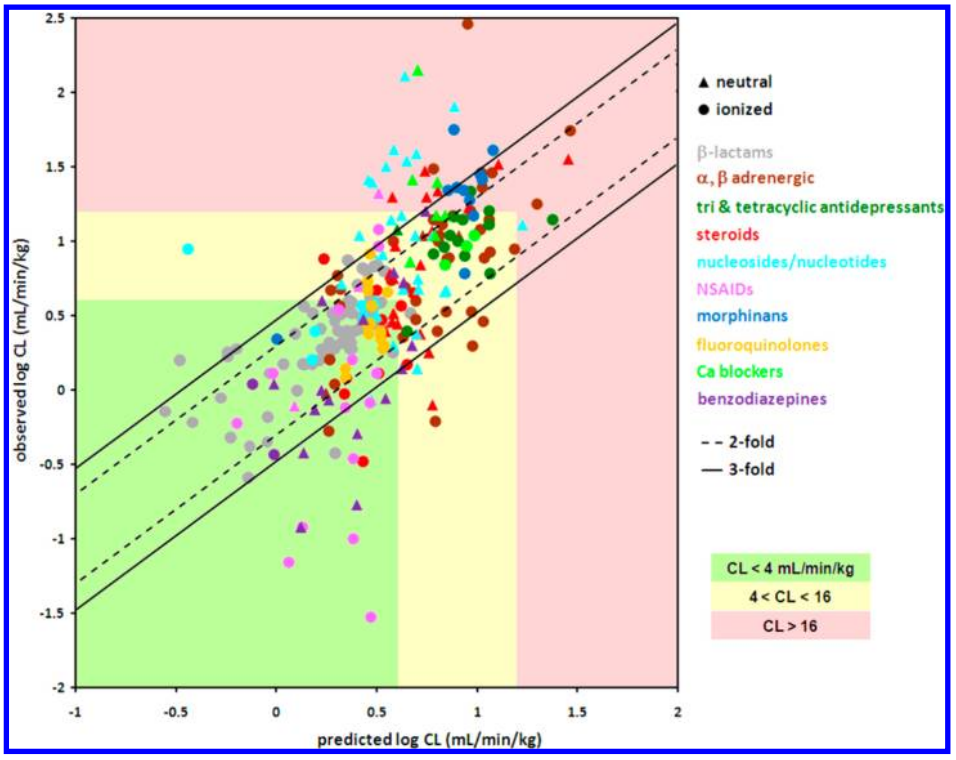


Figure 2. ELASTICO observed vs predicted total human plasma clearance (logarithmic scale). The dots are colored according to the analog/therapeutic class the compounds belong to, while the shape identifies the neutral (triangle-up) and ionized at pH 7.5 (circle) compounds. The background color is green, yellow, or red indicating that CL is low (less than 4 mL/min/kg), medium (in between 4 and 16 mL/min/kg), or high (greater than 16 mL/min/kg), respectively.

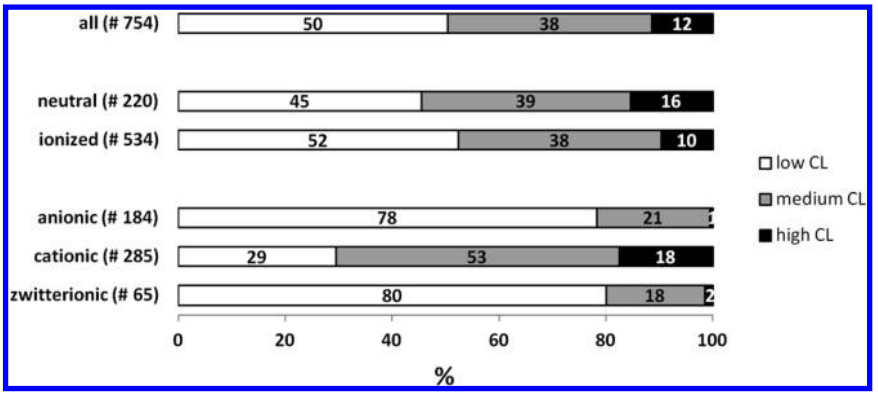


Figure 3. Distribution of ionized and neutral molecules based on low, medium, and high clearance (754 compounds). The bars show a higher percent of neutral compounds with high clearance. The zwitterionic and anionic classes show similar distribution with 80% and 78% of compounds with low clearance and only 2% and 1% with high clearance, respectively.

In Figure 3, we reported the distribution of all 754 compounds according to their ionization state based on low, medium, and high clearance. Overall, the 50%, 38%, and 12% of compounds have low, medium, or high clearance, respectively. The ionized compounds show a similar distribution, while the neutral have a greater percentage of high clearance (16%) and a lower percentage of low-clearance (45%). It is interesting to note the majority of compounds belonging to anionic (as the NSAIDs) and zwitterionic classes have a low clearance (78% and 80%, respectively) and only 1−2% has a high clearance. This could be explained with the higher tendency of zwitterionic and anionic compounds to reside within plasma due to their high hydrophilicity or high plasma protein binding and consequent lower membrane permeation into clearance organs. On the contrary, a lower percentage of cationic compounds show a low clearance with a value of 29% versus a 53% and 18% of compounds with medium and high clearance, respectively. These molecules show generally a higher distribution due to a higher affinity of basic moieties with the biological membranes (morphinans, for instance).

The ELASTICO validation approach has also been applied using a similarity approach as described in the Methods section. The best results are reported in Table S3 as Supporting Information, and they do not show any improvement compared with the PLS model with an overall GMFE of 2.3 and the 53% and 72% of prediction within 2- and 3-fold, with a maximum fold error of 133.

Descriptor Interpretation. A linear PLS model built combining physicochemical descriptors with structural fragments increases the overall accuracy of prediction, and it can

dx.doi.org/10.1021/ci300155y | J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

Table 2. Twenty Most Important Descriptors[a]

Variable #	Name / SMARTS notation	Definition / Representation	PLS scaled coefficient
1	AI7.0	Anionic fraction at pH 7. It calculated as the sum of fractions x number of charges of all anionic species at pH 7	-0.160
2	[cH](cc)c(S)c		-0.108
3	lgD10	1-octanol/water diffusion coefficient calculated at pH 10	+0.104
4	%FU4	% of neutral species at pH 4	-0.088
5	%FU10	% of neutral species at pH 10	+0.086
6	PSA	Polar Surface Area	-0.084
7	[C](O)(=O)C(C)O		-0.083
8	[NH](C(N)=O)S(c)(=O)=O		-0.078
9	[O](CC)C(C)C		-0.078
10	[CH2](N=C)C(N)=O		-0.076
11	[N](CC)=C(c)c		-0.076
12	[CH](CC)=CC		-0.074
13	[c](O)(cc)c(O)c		+0.073
14	[CH](c)(C)O		+0.073
15	[c](Cl)(cc)cc		-0.073
16	[c](C(c)=N)(cc)cc		-0.070
17	[S](=O)(=O)(Nc)c(c)c		-0.065
18	DRACAC	The maximum conformational area of the pharmacophoric triplet between hydrophobic (DR) and two H-bond acceptor (AC) points	-0.064
19	[O]1C(C)CCC1n		+0.064
20	[CH2](C)c		+0.064

[a]See the Supporting Information for the complete list of 63 descriptors of the PLS model.

provide important chemical indications for the optimization of drug candidate in drug design.

In Table 2, the 20 most important physicochemical descriptors and fragments are reported with the values of the PLS scaled coefficient (see the Supporting Information for a complete list of all 63 descriptors and fragments used to build the model). A negative or positive coefficient indicates that a higher value of that physicochemical descriptor (or the presence of a fragment) contributes to respectively reduce or increase clearance.

The final physicochemical descriptors used from the PLS model mainly characterize the ionization state and/or hydrophilicity/lipophilicity of molecules. The most important negative coefficients belongs to AI7.0, %FU4, PSA, and DRACAC physicochemical descriptors, where AI7.0 and % FU4 represent the fraction of anionic species at pH 7 and the percentage of unionized compounds at pH 4, respectively. The PSA is the polar surface area and the pharmacophoric DRACAC is the maximum conformational area of triangle generated from the triplet of distances between hydrophobic (DR) and two H-bond acceptor (AC) points. Other pharmacophoric triplets appear among the complete list of 63 descriptors of model, and all include only hydrophobic and/or H-bond acceptor points as DRACAC and ACACAC. Generally, these descriptors primarily identify the bigger and hydrophilic compounds such as antifungal compounds (i.e., anidulafungin, caspofungin, micafungin) or peptide antibiotics (i.e., vancomycin and daptomycin) and their propensity to show a high affinity with plasma. It is interesting to note that, similarly to Wajima's equation,[2] which combines rat and dog clearance values with molecular weight and number of H-bond acceptors, the triplets of distances including the H-bond donor points do not show improvement in our model. On the other hand, the most important positive coefficients belong to lgD10 and % FU10, representing the 1-octanol/water distribution coefficient and fraction unionized calculated at pH 10, respectively. These descriptors mainly identify the basic compounds generally showing a higher permeability due to higher membrane affinity.

The analysis of structural fragments shows that the aromatic sulfones, sulfoxide, sulfonamides, represented by SMARTS strings 2, 8, and 17 in Table 2, characterize mainly compounds with low clearance, perhaps because of a higher hydrophilicity

dx.doi.org/10.1021/ci300155y | J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

of these chemical groups[35] that increases the affinity of compounds with the plasma. Alternatively sulfoxides and sulfones could be considered "pre-metabolized" sulfides and thus no longer prone to oxidative metabolism. Likewise the α-hydroxy carboxylate (variable 7 in Table 2), mainly identifies the high hydrophilic glucuronic acid chemical group. The negative coefficient of variable 15 confirms the likely function of chlorine atoms in protecting phenyl groups from metabolism, and it is interesting that, after statistical optimization of the model, no groups with fluorine were present in the final list of 63 variables, maybe leading to the hypothesis that the chlorine is better than fluorine to protect aromatic groups. Other important fragments, not reported in Table 2 are those accounting for ester groups (see variables 28, 29, 44, and 51 in the Supporting Information). The positive coefficients for these fragments agree with the fact the ester group is often enzymatically hydrolyzed by ubiquitous esterases present in both plasma and tissues.

The analysis of fragments does not always yield a chemical interpretation of a metabolic process, but points toward a certain class of analogs with a specific common scaffold. For instance, the SMARTS strings 10 and 11 represent exclusively the analog class of benzodiazepines that are characterized by carbon−nitrogen double bond. Another example is the fragment 19 in the Table 2 that represents the aliphatic 5-membered ring present only in the nucleosides and nucleotides. This may explain the poor accuracy of the ELASTICO validation approach for the nucleosides/nucleotides and benzodiazepines therapeutic classes, when fragments only present in specific classes are not otherwise present in the recast general model now lacking the needed fragments.

Variables 14 and 20 indicate other fragments with a positive coefficient value. They represent benzylic moieties, commonly considered a very labile group toward oxidation in hepatic metabolism.[36]

The specific and capillary application to a given compound (or compounds) of a set of 63 diverse descriptors may not be feasible as there are opposing effects, although we recognize and discuss some of the fragments and continuum descriptors in some detail (e.g., sulfoxide and sulfones in terms of polarity and premetabolism of sulfides). And the analysis shows that fragments and physicochemical properties are context dependent and any extrapolation or "singling out" may be misleading, in a complex reality such as clearance and with metabolic switching always lurking. However, for instance, one potential misconception we dispel is the use of fluorine as almost a panacea in blocking sites of metabolism.

In silico vs in vivo Comparison. We also compared the PLS in silico model with several in vivo methods. The HBF−monkey proportionality with the hepatic blood flow correction (HBF),[3] the fu (fraction unbound) corrected intercept method (FCIM) applied using the rat, dog, and monkey species,[7] and a new multiple linear regression (MLR) equation based on rat and dog clearance data have been recently shown to be highly predictive in vivo methods.[14] The prediction accuracy of the present in silico method was also tested utilizing the in vivo method for all available compounds. We removed, in turn, all the N compounds shared by the full in silico compound set and each of the in vivo scaling methods to be compared and built a new in silico method with the remaining compounds in each case. Then, each in silico model, recast without any of the compounds available for the in vivo scaling method to be compared was tested against the latter for performance.

Table 3 reports the statistical results for all compounds, including those neutral and ionized, using both the monkey−HBF proportionality method and the newly developed in silico PLS model across the 131 compounds with monkey PK data. Overall, the in silico model does not perform far from the monkey−HBF proportionality approach with a GMFE of 2.1 vs 1.9 and a percentage of prediction within 2-fold of 55% vs 60%, respectively, for the PLS model and the in vivo method. The in silico method is inferior if the 3-fold threshold is considered with a 76% vs 84% of compounds predicted within the latter threshold, but the usefulness of such threshold is debatable and the maximum fold error is the same for both methods at 23.

Table 3. In silico vs in vivo (PLS Model vs Monkey−Human Proportionality)

in vivo monkey HBF	all	neutral	ionized	anionic	cationic	zwitterionic
no. compounds	*131*	*34*	*97*	*41*	*32*	*24*
GMFE	**1.9**	**1.9**	**2.0**	**2.2**	**1.9**	**1.6**
% < 2-fold	**60**	**59**	**60**	**56**	**56**	**71**
% < 3-fold	**84**	**82**	**85**	**80**	**81**	**96**
max fold error	23	8	23	23	7	4
in silico PLS model	**all**	**neutral**	**ionized**	**anionic**	**cationic**	**zwitterionic**
no. compounds	*131*	*34*	*97*	*41*	*32*	*24*
GMFE	**2.1**	**2.3**	**2.1**	**2.4**	**2.2**	**1.5**
% <2-fold	**55**	**41**	**60**	**54**	**53**	**79**
% <3-fold	**76**	**71**	**78**	**63**	**84**	**96**
max fold error	23	14	23	17	23	4

Similarly, Table 4 reports the performance comparison of the FCIM method using data in rat, dog, and monkey, with the in silico model for all 69 compounds with data available. Although these 69 compounds may not be a true representation of either the 131 with monkey data (obviously the 69 are included in the 131 compounds) or all 754 compounds, the in silico model shows a superior or equal performance in all cases. Overall the in silico model yields a GMFE of 1.8 vs 1.9 for the FCIM method and a percentage of prediction within a 2-fold error for 70% vs 62% of the compounds recorded for the FCIM method. Both methods show a somewhat decreased performance for anionic compounds, but the in silico model shows a very good prediction in particular for zwitterionic compounds (92% within 2-fold error vs 69% for the FCIM) and, at least for this smaller data set, even with neutral compounds.

Table 4. In silico vs in vivo (PLS Model vs FCIM)

in vivo FCIM	all	neutral	ionized	anionic	cationic	zwitterionic
no. compounds	*69*	*15*	*54*	*22*	*19*	*13*
GMFE	**1.9**	**1.8**	**2.0**	**2.5**	**1.6**	**1.8**
% <2-fold	**62**	**60**	**63**	**45**	**79**	**69**
% <3-fold	**78**	**73**	**80**	**73**	**84**	**85**
max fold error	47	8	47	47	4	5
in silico PLS model	**all**	**neutral**	**ionized**	**anionic**	**cationic**	**zwitterionic**
# compounds	*69*	*15*	*54*	*22*	*19*	*13*
GMFE	**1.8**	**1.9**	**1.8**	**2.4**	**1.6**	**1.4**
% <2-fold	**70**	**60**	**72**	**59**	**74**	**92**
% <3-fold	**84**	**87**	**83**	**59**	**100**	**100**
max fold error	16	6	16	16	3	3

dx.doi.org/10.1021/ci300155y | J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

The same kind of comparison of PLS model was then performed for a recently proposed in vivo MLR method.[14] This method has performed quite well in the prediction of human clearance, and it does so in the absence of monkey data, relying solely on the rat and dog clearance according to the following equation, with clearance expressed in mL/min/kg:

$$\log_{10}(\text{CL human}) = 0.4\log_{10}(\text{CL rat}) + 0.4\log_{10}(\text{CL dog}) - 0.4$$

Table 5 reports the prediction accuracy of the present in silico PLS model and of the in vivo MLR model for all 189 compounds with rat and dog clearance common to both data sets. Overall the in silico model shows a GMFE of 2.1 vs 2.2 for the MLR method and similar predictive performances with 59% vs 60% for the 2-fold and 76% vs 80% for the 3-fold, respectively.

Table 5. In silico vs in vivo (PLS Model vs MLR Rat−Dog)

in vivo MLR rat−dog	all	neutral	ionized	anionic	cationic	zwitterionic
no. compounds	*189*	*46*	*143*	*42*	*73*	*28*
GMFE	**2.2**	**2.9**	**2.0**	**2.3**	**2.0**	**1.5**
% <2-fold	**60**	**41**	**66**	**57**	**62**	**89**
% <3-fold	**80**	**65**	**85**	**76**	**86**	**96**
max fold error	98	60	98	98	39	3
in silico PLS model	**all**	**neutral**	**ionized**	**anionic**	**cationic**	**zwitterionic**
no. compounds	*189*	*46*	*143*	*42*	*73*	*28*
GMFE	**2.1**	**2.8**	**1.9**	**2.2**	**2.0**	**1.5**
% <2-fold	**59**	**43**	**64**	**57**	**60**	**82**
% <3-fold	**76**	**63**	**80**	**64**	**82**	**96**
max fold error	35	35	24	15	24	5

The analysis of results according to the ionization state confirms the accuracy of the in silico model to predict ionized compounds in particular cationic and zwitterionic molecules showing the same or even higher accuracy than for the in vivo MLR rat−dog method. Unfortunately both methods seem to be inadequate for neutral compounds showing a very high GMFE (2.8 and 2.9) and a low percentage of compounds predicted within 2-fold (43% and 41%) and 3-fold (63% and 65%).

It may be argued that it would be better to compare all methods on the same data set; therefore in Table 6, we report the prediction results of the in silico model with all three in vivo methods based on the same 69 compounds that all approaches share, although they are not representative of the entire data set. In this case, the in silico model remarkably shows a very good performance with the best GMFE, maximum fold error, and percentage of predicted within 2- and 3-fold. However it is important to note that unlike FCIM and monkey HBF, both the in silico model and the in vivo MLR rat−dog method are training set dependent. Therefore it is possible that analogs of the 69 compounds, present among remaining 685, may favorably bias the prediction.

Table 6. Overall in silico vs in vivo with the Same Compounds

in silico vs in vivo	in silico PLS	monkey HBF	FCIM	MLR rat−dog
no. compounds	*69*	*69*	*69*	*69*
GMFE	**1.8**	**1.9**	**1.9**	**2.1**
% <2-fold	**70**	**62**	**62**	**59**
% <3-fold	**84**	**84**	**78**	**83**
max fold error	16	23	47	98

Finally a comparison of the qualitative prediction of the in silico model with each of three in vivo methods is shown in Table 7, where the binning capacities of methods to predict low, medium, or high clearance compounds (defined as previously discussed) are reported based on the maximum number of data available for each in vivo method. With the exception of the 81% value for the in silico model against the FCIM (69 compounds), all methods yield the same total percent of binning confidence set at approximately 70%, but the in silico model shows a particular strength in predicting low-clearance compounds (90%, 90%, and 84% vs 77% for monkey−HBF, 79% for FCIM, and 83% for MLR rat−dog, respectively), while it shows the same capability to predict medium-clearance compounds as well as in vivo methods (60% vs 62% with the monkey−HBF, 75% vs 73% with the FCIM, and 69% vs 67% with the MLR rat−dog). It is very interesting the fact that the monkey−HBF is the only method with a very good accuracy of high-clearance prediction (79%) while all other in silico and in vivo methods do not do better than 25%, confirming the predictive reliability of the monkey species in preclinical drug discovery.

The performance of the new in silico model has been compared with in vivo methods in the prediction of clearance of 13 internal compounds in phase I and the results are reported in the Table 8. Again, the in silico model shows a competitive prediction by yielding a GMFE of 2.1 vs a value of 2.2, 2.0, and 1.8 for monkey−HBF, FCIM, and MLR rat−dog, respectively. However, it should be noted that it has not been possible to compare the methods on all 13 compounds because data for all preclinical species were not available for all compounds and sometimes the FCIM method was applied using only two species (rat−dog or rat−monkey). Therefore, while an in silico method can predict clearance solely based on chemical structure, and that is an obvious strength, we could not truly compare it across a full matrix of methods and compounds.

CONCLUSIONS

The prediction of human clearance is a lofty goal and fraught with challenges, whether it is based on in silico, in vitro, or in vivo methods and the comparative analysis, reported here as part of the validation efforts, does illustrate those aspects. More specifically physicochemical descriptors were shown as by other authors to be able to predict the clearance of only ionized molecules within a useful accuracy, but they seem inadequate when applied toward the prediction of clearance of neutral compounds. We have explored the application of physicochemical descriptors, and we have observed a similar performance by using several linear and nonlinear statistical tools such as PLS, RF, similarity, and rule-based methods.

The in silico PLS model built with the addition of structural fragments better represents the biotransformation processes, and it clearly represents an improvement for the prediction of human clearance for both ionized and neutral compounds, able to compare favorably with the best in vivo methods.

The in silico model shows an overall comparable or better accuracy than the MLR rat−dog in vivo method with a predictive accuracy within 2-fold for the ionized compounds in particular cationic and zwitterionic, the latter even with a

dx.doi.org/10.1021/ci300155y | J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

Table 7. In silico vs in vivo Binning Capacity

% bin capacity	in silico PLS	in vivo monkey−HBF	in silico PLS	in vivo FCIM	in silico PLS	in vivo MLR rat−dog
all	71	72	81	74	71	69
low	90	77	90	79	84	83
medium	60	62	75	73	69	67
high	14	79	25	25	19	10

Table 8. In silico vs in vivo with Internal Compounds

in silico vs in vivo	in silico PLS	monkey HBF	FCIM[a]	MLR rat−dog
no. compounds	*13*	*7*	*10*	*7*
GMFE	**2.1**	**2.2**	**2.0**	**1.8**
max fold error	6	12	5	3

[a]Applied using all three rat, dog, and monkey species or only two (rat−dog or rat−monkey).

GMFE of 1.5, while it tends to overestimate the clearance of anionic compounds, but still qualitatively well-predicted as low. Although an improvement has been achieved, the neutral compounds can be predicted very well only using the in vivo methods based on the monkey clearance corrected for hepatic blood flow and in general, whenever a compound shows a high clearance, that the in silico model tends to underestimate its clearance value. On the contrary, since the in silico model seldom underestimates the low-clearance compounds with a clearance less than 4 mL/min/kg, it yields a very good binning capacity to identify low-clearance compounds.

This in silico model built using the PLS linear regression and based on the combined use of interpretative physicochemical and structural descriptors should be useful in drug design for the optimization of candidate compounds in addition to predicting pharmacokinetics before administration to humans.

METHODS

Data Set. All human total plasma clearance values were taken from published work and in part were reported by Obach et al.[37] and Berellini et al.[32] Only intravenous data were accepted, often checking the analytical procedure adopted by the respective authors, doses and other parameters that could influence the results. In the case of several references a weighted average was taken based on the number of subjects. In some cases, we digitized reported plots via DigitizeIt version 1.5.7 (available at www.digitizeit.de) and performed the pharmacokinetic calculations using WinNonLin version 5.2 (Pharsight Corporation, Cary NC). The list of references and complete data set, together with our comments and annotations, is provided as Supporting Information.

A total of 754 compounds have been used after the exclusion of artesunate, carboplatin, gadoversetamine (because of failure in the fragment generation, explained in the Descriptors section) and 7-hydroxystaurosporine, a compound that, due to its extensive and species-dependent plasma protein binding, has already been reported to be a systematic outlier in clearance prediction using in vivo data.[38]

The data set is comprised of 220 neutral and 534 ionized compounds at pH 7.5 (184 anionic, 285 cationic, 65 zwitterionic) and many compounds are identified according to their therapeutic/structural class. Only classes with at least 10 compounds were considered and reported. The complete information about the data set is reported as Supporting Information.

Descriptors. The VolSurf+ physicochemical descriptors[39,40] and anionic index, calculated according to pKas predicted by MoKa (version 1.0, Molecular Discovery Ltd., UK),[41] in combination with structural fragments (represented in form of SMARTS strings[42]) were used as variables to build several models. The fragments were generated starting from each atom of a molecule and accounting for all neighboring atoms up to a three-bond distance. Due to the high number of all combinations of possible fragments (>4000), only the fragments appearing at least 3 times were taken into account (∼1500). All compounds were coded as SMILES strings[43] as input for the calculations.

Modeling. Several models are been built to correlate the logarithm of human clearance using only physicochemical descriptors or in combination with structural fragments. Linear methods, such as partial least-squares (PLS),[29] and nonlinear such as random forest (RF)[30] (version 4.6.6 included in the R package 2.14.1), rule-based[31] (Cubist) and the similarity approach have been used as statistical regression tools. The PLS models have been optimized by following the same approach used to predict the volume of distribution reported in previous work.[32] The similarity approach is based on searching the nearest neighbors according to the Euclidean distance on the autoscaled chemical space generated from descriptors. The predicted values are the result of the average of the clearance of selected nearest neighbors. Several combinations of filters have been applied to optimize the selection of nearest neighbors such as fixing a maximum number of nearest neighbors and/or maximum Euclidean distance. The numbers of nearest neighbors tested to define the best maximum number of neighbors to be considered are 3, 7, 10, 20, 50, and 100. The values of 1.5- and 2-fold of the first nearest neighbor distance were used to optimize the maximum Euclidean distance within a range where all nearest neighbors have to be taken into account.

A PLS model was finally built to linearly correlate the physicochemical descriptors and structural fragments with the following steps: (i) all fragments mentioned above (>4000) were examined for frequency of occurrence and those which were not present at least three times across the data set were eliminated; (ii) using a jack-knife process available within SIMCA-P+[44,45] (version 12.0.1, Umetrics, Umea, Sweden), we decreased the remaining ∼1500 fragments and physicochemical descriptors to the final 54 and 9, respectively (63 total). The jack-knifed confidence intervals in the coefficients plot were used to identify important and significant variables, discarding the variables showing an absolute value of confidence interval higher than the coefficient value.[46] The final optimized PLS model is based only on the selected 63 variables at the second component.

Validation. The accuracy of prediction for all methods has been validated comparing the geometric mean fold error (GMFE), percent of predicted within 2- and 3-fold, and maximum fold error:

dx.doi.org/10.1021/ci300155y | J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

$$\text{GMFE} = 10^{\left[\sum |\log_{10}(\text{predicted}/\text{observed})|/N\right]}$$

$$\text{fold error} = 10^{|\log_{10}(\text{predicted}/\text{observed})|}$$

$$\text{max fold error} = 10^{\max[|\log_{10}(\text{predicted}/\text{observed})|]}$$

A GMFE within 2 and 60% of compounds predicted within 2-fold were been considered acceptable thresholds for a good prediction accuracy as reported in a recent review comparing several in vivo methods.[14]

The out-of-bag (OOB) error included in the RF has been used to validate the RF models (the default parameters used were ntree = 500, number of descriptors = sqrt (N), and OOB = $^2/_3$), while the leave-one-out (LOO) cross-validation approach has been used for all other models as criteria to select the models showing higher performance for the ELASTICO validation. ELASTICO was a more robust cross-validation method used for accuracy of prediction purposes of the better performing in silico models such as PLS and PLS-similarity. This method consists to classify compounds according to their structural and/or therapeutic class and the accuracy of prediction was also evaluated according to their ionization class (neutral, anionic, cationic, and zwitterionic). The classification of compounds in neutral, anionic, cationic, and zwitterionic has been defined during the VolSurf+ structure loading step, according to ionization state of the major species of a compound at pH 7.5.

Finally, the better performing in silico PLS model was compared with the human−monkey proportionality with hepatic blood flow correction proposed by Ward and Smith,[3] the FCIM,[7] and the MLR rat−dog (a multiple linear regression using only rat and dog in vivo data).[14] These methods were recently identified to be quite accurate in vivo methods to predict the human clearance.[14] To make the comparison meaningful, we removed, in turn, all the N compounds shared by the full in silico compound set and each of the in vivo scaling methods to be compared and built a new in silico method with the remaining compounds in each case. Then each in silico model, recast without any of the compounds available for the in vivo scaling method to be compared, was tested against the latter for performance.

■ ASSOCIATED CONTENT

Supporting Information

The complete table with human plasma clearance and relative references and annotations. The list of final 63 physicochemical descriptors and structural fragments in SMARTS format with relative PLS coefficient values of the in silico PLS model. The table with the results of ELASTICO validation of the similarity model. This material is available free of charge via Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (617) 871-4003. Fax: +1 (617) 871-3078. E-mail: franco.lombardo@novartis.com.

Present Address

‡Epizyme, Inc., 325 Vasser Street, Cambridge MA, USA.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully thank Dr. P. Ertl (CADD group at NIBR, Novartis Pharma AG, Basel, Switzerland) for an in house program used to automatically extract the SMARTS strings of fragments from each structure.

■ ABBREVIATIONS:

PK, pharmacokinetic; HBF, hepatic blood flow; MLR, multiple linear regression; PLS, partial least-squares; RF, random forest; *k*NN, *k*-nearest-neighbors; GMFE, geometric mean fold error; SMILES, simplified molecular input line entry system; SMARTS, smiles arbitrary target specification; LOO, leave-one-out; OOB, out-of-bag; LCO, leave class out; ELASTICO, enhanced leave analog-structural therapeutic ionization class out

■ REFERENCES

(1) Dedrick, R. L. Animal scale-up. *J. Pharmacokinet. Biopharm.* **1973**, *1*, 435−461.

(2) Wajima, T.; Fukumura, K.; Yano, Y.; Oguma, T. Prediction of human clearance from animal data and molecular structural parameters using multivariate regression analysis. *J. Pharm. Sci.* **2002**, *91*, 2489−2499.

(3) Ward, K. W.; Smith, B. R. A comprehensive quantitative and qualitative evaluation of extrapolation of intravenous pharmacokinetic parameters from rat, dog, and monkey to humans. I. Clearance. *Drug Metab. Dispos.* **2004**, *32*, 603−611.

(4) Caldwell, G. W.; Masucci, J. A.; Yan, Z.; Hageman, W. Allometric scaling of pharmacokinetic parameters in drug discovery: Can human CL, Vss and t1/2 be predicted from in-vivo rat data? *Eur. J. Drug Metab. Pharmacokinet.* **2004**, *29*, 133−143.

(5) Nagilla, R.; Ward, K. W. A comprehensive analysis of the role of correction factors in the allometric predictivity of clearance from rat, dog, and monkey to humans. *J. Pharm. Sci.* **2004**, *93*, 2522−2534.

(6) Jolivette, L. J.; Ward, K. W. Extrapolation of human pharmacokinetic parameters from rat, dog, and monkey data: Molecular properties associated with extrapolative success or failure. *J. Pharm. Sci.* **2005**, *94*, 1467−1483.

(7) Tang, H.; Mayersohn, M. A novel model for prediction of human drug clearance by allometric scaling. *Drug Metab. Dispos.* **2005**, *33*, 1297−1303.

(8) Evans, C. A.; Jolivette, L. J.; Nagilla, R.; Ward, K. W. Extrapolation of preclinical pharmacokinetics and molecular feature analysis of "discovery-like" molecules to predict human pharmacokinetics. *Drug Metab. Dispos.* **2006**, *34*, 1255−1265.

(9) Mahmood, I.; Martinez, M.; Hunter, R. P. Interspecies allometric scaling. Part I: prediction of clearance in large animals. *J. Vet. Pharmacol. Therapeut.* **2006**, *29*, 415−423.

(10) Martinez, M.; Mahmood, I.; Hunter, R. P. Interspecies allometric scaling: prediction of clearance in large animal species: part II: mathematical considerations. *J. Vet. Pharmacol. Therapeut.* **2006**, *29*, 425−432.

(11) Tang, H.; Mayersohn, M. A global examination of allometric scaling for predicting human drug clearance and the prediction of large vertical allometry. *J. Pharm. Sci.* **2006**, *95*, 1783−1799.

(12) Mahmood, I. Role of Fixed Coefficients and Exponents in the Prediction of Human Drug Clearance: How Accurate Are the Predictions from One or Two Species. *J. Pharm. Sci.* **2009**, *98*, 2472−2493.

(13) Lave, T.; Chapman, K.; Goldsmith, P.; Rowland, M. Human clearance prediction: shifting the paradigm. *Expert Opin. Drug Metab. Toxicol.* **2009**, *5*, 1039−1048.

(14) Lombardo, F.; Waters, N. J.; Argikar, U.; Dennehy, M. K.; Zhan, J.; Gunduz, M.; Harriman, S. P.; Berellini, G.; Liric Rajlic, I.; Obach, R. S. Comprehensive Assessment of Human Pharmacokinetic Prediction Based on In Vivo Animal Pharmacokinetic Data. Part 2: Clearance. *J. Clin. Pharmcol.* **2012**, DOI: 10.1177/0091270012440282.

(15) Houston, J. B. Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. *Biochem. Pharmacol.* **1994**, *47*, 1469−1479.
(16) Iwatsubo, T.; Hirota, N.; Ooie, T.; Suzuki, H.; Shimada, N.; Chiba, K.; Ishizaki, T.; Green, C. E.; Tyson, C. A.; Sugiyama, Y. Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. *Pharmacol. Ther.* **1997**, *73*, 147−171.
(17) Obach, R. S.; Baxter, J. G.; Liston, T. E.; Silber, B. M.; Jones, B. C.; Macintyre, F.; Rance, D. J.; Wastall, P. The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 46−58.
(18) Howgate, E. M.; Rowland-Yeo, K.; Proctor, N. J.; Tucker, G. T.; Rostami-Hodjegan, A. Prediction of In Vivo Drug Clearance From In Vitro Data. I: Impact of Inter-Individual Variability. *Xenobiotica* **2006**, *36*, 473−497.
(19) Shiran, M. R.; Proctor, N. J.; Howgate, E. M.; Rowland-Yeo, K.; Tucker, G. T.; Rostami-Hodjegan, A. Prediction of Metabolic Drug Clearance in Humans: In Vitro-In Vivo Extrapolation vs Allometric Scaling. *Xenobiotica* **2006**, *36*, 567−580.
(20) Nikolic, K.; Agababa, D. Prediction of Hepatic Microsomal Intrinsic Clearance and Human Clearance Values for Drugs. *J. Mol. Graphics Modell.* **2009**, *28*, 245−252.
(21) Li, H.; Sun, J; Sui, X.; Liu, J.; Yan, Z.; Liu, X.; Sun, Y.; He, Z. First Principle, Structure-Based Prediction of Hepatic Metabolic Clearance Values in Human. *Eur. J. Med. Chem.* **2009**, *44*, 1600−1606.
(22) Yang, X.; Gandhi, Y. A.; Duignan, D. B.; Morris, M. E. Prediction of Biliary Excretion in Rats and Humans Using Molecular Weight and Quantitative Structure-Pharmacokinetic Relationships. *AAPS J.* **2009**, *11*, 511−525.
(23) Varma, M. V. S.; Feng, B.; Obach, R. S.; Troutman, M. D.; Chupka, J.; Miller, H. R.; El-Kattan, A. Physicochemical Determinants of Human Renal Clearance. *J. Med. Chem.* **2009**, *52*, 4844−4852.
(24) Paixao, P.; Gouveia, L. F.; Morais, J. A. G. Prediction of the In Vitro Intrinsic Clearance Determined in Suspensions of Human Hepatocytes by Using Artificial Neural Networks. *Eur. J. Pharm. Sci.* **2010**, *39*, 310−321.
(25) Obach, R. S. Predicting clearance in humans from in vitro data. *Curr. Top. Med. Chem.* **2011**, *11*, 334−339.
(26) Yap, C. W.; Li, Z. R.; Chen, Y. Z. Quantitative Structure-Pharmacokinetic Relationships for Drug Clearance by Using Statistical Learning Methods. *J. Mol. Graphics Modell.* **2006**, *24*, 383−395.
(27) Yu, M. J. Predicting Total Clearance in Humans from Chemical Structure. *J. Chem. Inf. Model.* **2010**, *50*, 1284−1295.
(28) Demir-Kavuk, O.; Bentzien, J.; Muegge, I.; Knapp, E.-W. DemQSAR: predicting human volume of distribution and clearance of drugs. *J. Comput.-Aided Mol. Des.* **2011**, *25*, 1121−1133.
(29) Wold, S.; Albano, C.; Dunn, W. J. III; Edlund, U.; Esbensen, K.; Geladi, P.; Hellberg, S.; Johansson, E.; Lindberg, W.; Sjöström., M. Multivariate Data Analysis in Chemistry. In *Chemometrics: Mathematics and Statistics in Chemistry*; Kowalski, B. R., Ed.; Reidel Publishing Company: Dordrecht, Holland, 1984; pp 17−95.
(30) Svetnik, V.; Liaw, A.; Tong, C.; Culberson, J. C.; Sheridan, R. P.; Feuston, B. P. Random Forest: a classification and regression tool for compound classification and QSAR modeling. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 1947−1958.
(31) *Cubist*, release 2.06. RuleQuest Research; http://www.rulequest.com/cubist-info.html (accessed September 2009).
(32) Berellini, G.; Springer, C.; Waters, N.; Lombardo, F. In Silico Prediction of Volume of Distribution in Human using Linear and Non-Linear Models on a 669 Compound Data Set. *J. Med. Chem.* **2009**, *52*, 4488−4495.
(33) Lombardo, F.; Obach, R. S.; Shalaeva, M. Y.; Gao, F. Prediction of human volume of distribution values for neutral and basic drugs. 2. Extended data set and leave-class-out statistics. *J. Med. Chem.* **2004**, *47*, 1242−1250.
(34) Lombardo, F.; Obach, R. S.; DiCapua, F. M.; Bakken, G.; Lu, J.; Potter, D. M.; Gao, F.; Miller, M. D.; Zhang, Y. A hybrid mixture discriminant analysis-random forest computational model for the prediction of volume of distribution in human. *J. Med. Chem.* **2006**, *49*, 2262−2267.
(35) Caron, G.; Gaillard, P.; Carrupt, P.-A.; Testa, B. Lipophilicity Behavior of Model and Medicinal Compounds Containing a Sulfide, Sulfoxide, or Sulfone Moiety. *Helv. Chim. Acta* **1997**, *80*, 449−462.
(36) Uetrecht, J. P.; Trager, W. Oxidation Pathways and the Enzymes That Mediate Them. In *Drug Metabolism: Chemical and Enzymatic Aspects*; Informa Healthcare USA, Inc: New York, 2007; pp 33−108.
(37) Obach, R. S.; Lombardo, F.; Waters, N. J. Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 compounds. *Drug Metab. Dispos.* **2008**, *36*, 1385−1405.
(38) Tang, H.; Mayersohn, M. On the Observed Large Inter-Species Over-Prediction of Human Clearance ("Vertical Allometry") of UCN-01: Further Support for a Proposed Model Based Upon Plasma Protein Binding. *J. Clin. Pharmacol.* **2006**, *46*, 398−400.
(39) *VolSurf+*, version 1.0.4; Molecular Discovery: London, UK; http://www.moldiscovery.com/soft_vsplus.php (accessed January 2012).
(40) Fortuna, C. G.; Barresi, V.; Berellini, G.; Musumarra, G. Design and Synthesis of *trans* 2-(furan-2-yl)vinyl heteroaromatic iodides with antitumour activity. *Bioorg. Med. Chem.* **2008**, *16*, 4150−4159.
(41) Milleti, F.; Storchi, L.; Sforna, G.; Cruciani, G. New and Original pKa Prediction Method Using Grid Molecular Interaction Fields. *J. Chem. Inf. Model.* **2007**, *47*, 2172−2181.
(42) SMARTS notation. http://www.daylight.com/dayhtml/doc/theory/theory.smarts.html (accessed January 30, 2012).
(43) SMILES notation. http://www.daylight.com/dayhtml/doc/theory/theory.smiles.html (accessed January 30, 2012).
(44) *SIMCA-P+*, version 12.0; Umetrics; http://www.umetrics.com/simca (accessed January 30, 2012).
(45) Wold, S.; Sjostrom, M. SIMCA: a method for analyzing chemical data in terms of similarity and analogy. In *Chemometrics: Theory and Application*; Kowalski, B. R., Ed.; ACS Symposium Series; American Chemical Society: Washington DC, 1977, pp 243−282.
(46) Efron, B. Bootstrap methods: Another look at the jackknife. *Ann. Stat.* **1979**, *1*, 1−26.