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Quantitative Structure−Activity Relationship Models of Clinical Pharmacokinetics: Clearance and Volume of Distribution

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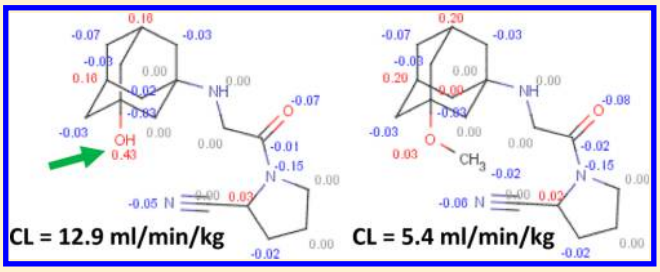
Supporting Information

ABSTRACT: Reliable prediction of two fundamental human pharmacokinetic (PK) parameters, systemic clearance (CL) and apparent volume of distribution (Vd), determine the size and frequency of drug dosing and are at the heart of drug discovery and development. Traditionally, estimated CL and Vd are derived from preclinical in vitro and in vivo absorption, distribution, metabolism, and excretion (ADME) measurements. In this paper, we report quantitative structure−activity relationship (QSAR) models for prediction of systemic CL and steady-state Vd (Vdss) from intravenous (iv) dosing in humans. These QSAR models avoid uncertainty associated with preclinical-to-clinical extrapolation and require two-dimensional structure drawing as the sole input. The clean, uniform training sets for these models were derived from the compilation published by Obach et al. (*Drug Metab. Disp.* **2008**, *36*, 1385−1405). Models for CL and Vdss were developed using both a support vector regression (SVR) method and a multiple linear regression (MLR) method. The SVR models employ a minimum of 2048-bit fingerprints developed in-house as structure quantifiers. The MLR models, on the other hand, are based on information-rich electro-topological states of two-atom fragments as descriptors and afford reverse QSAR (RQSAR) analysis to help model-guided, in silico modulation of structures for desired CL and Vdss. The capability of the models to predict iv CL and Vdss with acceptable accuracy was established by randomly splitting data into training and test sets. On average, for both CL and Vdss, 75% of test compounds were predicted within 2.5-fold of the value observed and 90% of test compounds were within 5.0-fold of the value observed. The performance of the final models developed from 525 compounds for CL and 569 compounds for Vdss was evaluated on an external set of 56 compounds. The predictions were either better or comparable to those predicted by other in silico models reported in the literature. To demonstrate the practical application of the RQSAR approach, the structure of vildagliptin, a high-CL and a high-Vdss compound, is modified based on the atomic contributions to its predicted CL and Vdss to propose compounds with lower CL and lower Vdss.



1. INTRODUCTION

With a high rate of attrition in the clinic, drug discovery and development is a cost- and labor-intensive endeavor. The average rate for candidates entering phase I to become a commercial drug is around 11% with 39% of them failing in clinical development due to poor pharmacokinetic (PK) and absorption, distribution, metabolism, and excretion (ADME) properties.[1] In a competitive marketplace, successful drugs must have desirable pharmacokinetic properties along with acceptable safety and activity profiles.

High attrition in the clinic may partly be due to uncertainty in extrapolating ADME measurements in preclinical species to humans. Therefore, we ventured into making human PK predictions from quantitative structure−activity relationship (QSAR) models derived directly from the human PK data available in the literature. Application of QSAR models to assess human PK not only avoids preclinical to clinical extrapolation but also is significantly less resource-intensive as compared to traditional approaches of interspecies scaling and in vitro−in vivo extrapolation.[2−6] Furthermore, robust QSAR models with ability to make reasonably accurate predictions of human PK also have other significant benefits: First, they can impact early discovery by helping in prioritization of synthesis and testing of compounds with higher probability of desired PK. Second, they can help guide design of compounds with suitable human PK profile and can highlight structural moieties predominantly associated with poor or acceptable human PK properties.

Several QSAR models for prediction of preclinical ADME properties have been described[7−11] and have been utilized by drug discovery teams in the pharmaceutical industry, but QSAR models for prediction of clinical PK are uncommon. Wajima et al.[12] developed a hybrid model with 7 descriptors, including rat CL and dog CL, and 5 computed structural parameters to predict human CL for a small set of 68 drugs. This multiple linear regression (MLR) model returned a leave-one-out (LOO) Q^2 of 0.62 and performed better than the simple allometry method. Several attempts[13−16] have been made to also develop QSAR models for prediction of human

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bioavailability from varying sets of structurally diverse compounds with reasonable predictivity. Berellini et al.[17] applied linear and nonlinear methods to predict steady-state volume of distribution (Vdss) in humans within 2-fold for about 55% of an external test set. Yu[18] described application of a *k*-nearest neighbor (*k*NN) technique to predict total clearance (CL), albeit of a set of 462 compounds that did not include any neutral molecules. In the LOO test, their model returned an average fold error of 2.2. Our present work embodies two models each for prediction of intravenous (iv) CL and steady state Vd (Vdss) in humans.

Although these published computational models returned a performance comparable to that from much more resource-intensive methods, we wanted to investigate the subset of data that is representative of current drug discovery space. After briefly describing the steps for data harmonization, methods of structure quantification, and model development and validation in the following sections, we present a comparison of CL and Vdss observed in the clinic and those obtained from our in silico models for two data sets obtained after the development of the model.

2. METHODS

2.1. Data Set. The training sets for the present QSAR models were derived from the human iv CL and Vdss data compiled by Obach et al.,[19] in many instances through digitization of the published time−concentration plots. The available data were further cleaned and harmonized as per the following steps:

(1) Compounds not meeting an in-house discovery space filter (DSF) were excluded. The DSF is a set of 19 computed whole-molecule descriptors (Table 1), and a compound is flagged to fail DSF if five or more of these 19 descriptors are outside the specified range. The compounds that failed DSF included inorganics such as lithium carbonate, low molecular weight compounds such as hydroxyurea, phosphoric acids like foscarnet and fosfomycin, large cyclic antibiotics, and molecules with molecular weight above 1000, etc. In all, 87 compounds failed the in-house DSF.

(2) Three compounds (CAS nos. 160237-25-2, 192573-38-9, and 178908-09-3) were dropped as their structure could not be retrieved from Drug Bank.

(3) From nine pairs of compounds with different CAS registry numbers but identical 2D structure, one compound was kept and assigned CL value equal to the average of the pair.

(4) For the CL model, however, compounds with CL values more than 120% of the human hepatic blood flow (= 20 mL/(min kg)) were excluded for lack of reliability in the reported CL values.

Table 1. Whole Molecule Descritpors Defining Discovery Space Filter

molecular weight
molecular E-state
number of hydrogen bond donors
number of hydrogen bond acceptors
number of heavy atoms
number of rotatable bonds
number of rings
number of fused planar rings
aromaticity index
hydrophobic−hydrophilic atom ratio
polar surface area
solvent accessible surface area
solvation energy
kappa shape index order 1
kappa shape index order 2
kappa shape index order 3
cylindrical shape on *x*-axis
cylindrical shape on *y*-axis
cylindrical shape on *z*-axis

This harmonization led to a clean data set of 569 compounds for modeling Vdss and a set of 525 compounds for the CL model (median CL = 3.7 mL/(min kg) and mean CL = 5.8 mL/(min kg)). Both data sets are made available in the Supporting Information. The data were log transformed before modeling for two main reasons: first, to keep exclusion of leverage points to a minimum because the human data are expensive; second, to better satisfy the assumption of constant variance of errors that is required to make inferences more reliable from a multiple linear regression model. Further, without log transformation, the model could predict negative values for compounds with very low values of Vdss and CL, which are harder to infer.

2.2. Model Development. Two models each for prediction of iv CL and Vdss in humans were developed. One model employs the support vector regression (SVR) method,[20] and the second model uses a multiple linear regression (MLR) approach.

2.2.1. SVR Model. For the SVR model, each chemical structure was quantified with 77 fingerprints; each consisting of a minimum of 2048 bits representing the absence or presence of structural attributes, their counts, and binned cLogP values. The best among these fingerprints for any model was selected based on its performance in 50 cross-validation runs each of which was conducted by randomly selecting 60% of the available data for training the model and the remaining 40% for testing the model. The fingerprint giving the best average Q^2 in these 50 runs was selected for building the final model.

2.2.2. MLR Model. In contrast to the nonlinear approach of SVR, the second model uses a multiple linear regression (MLR) approach, which has been shown to predict Vdss reasonably well.[17] Our MLR model employs information-rich electro-topological states (E-states)[21] of two-atom fragments as descriptors. The in-house implementation of E-state computation generates E-state values of a parsimonious set of two-atom fragments to completely and uniquely define each molecule in the data set. The set of available E-state descriptors is subjected to multiple objective dimensionality reduction checks prior to building a preliminary QSAR model. The preliminary model is subjected to rigorous diagnostics[8] before declaring it suitable for predictive applications. This MLR models have been implemented in an in-house computational environment, VISDOM (visual in silico design of molecules). The algorithms in this environment allow calculation of prediction diagnostics to assess reliability of a prediction. These include lower (LO_95%) and upper (UP_95%) mean 95% confidence limits, applicability index (AppNdx) or variance factor,[22,23] number and extent of principal components outside the effective prediction domain[22,23] (DimOPD), number of model

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descriptors outside the range spanned by the training set (DescsOut), measure of new chemistry in the query structure in terms of new two-atom fragments (NewFrags), query coverage in terms of descriptors common to the query and the model (ContribDescs), and QSAR similarity. A brief description of each of these diagnostics is given below and discussed in Results and Discussion with examples from the test sets used to check models' performance.

2.2.2.1. Mean 95% Confidence Interval. For every LMR model in VISDOM, the lower end of the 95% confidence interval (LO_95%) and the upper end of the 95% confidence interval (UP_95%) of each prediction are reported. When confidence interval is broad (UP_95 to LO_95 ratio approaching 4), especially when the interval spans a significant range of the training set data and when other diagnostic flags are raised, one can expect a larger deviation between observed and predicted values.

2.2.2.2. Applicability Index (ApplNdx). This composite prediction diagnostic, also called variance factor, is a measure of the applicability of a model. The smaller the value of ApplNdx, the smaller the likelihood of model extrapolation. For each model, the maximum value of ApplNdx is determined by running the training set through the model and the model is considered not applicable to any query structure with ApplNdx value greater than this maximum value. The value of ApplNdx for the human iv CL model was observed to be 0.80.

2.2.2.3. Dimensions Outside Prediction Domain (DimOPD). The effective prediction domain[22] (PD) of each MLR model in VISDOM is defined in terms of the range of each principal component (dimension) obtained from model descriptors; total dimensions equal the number of model descriptors (89 for the human iv CL model and 85 for the Vdss model). DimOPD measures applicability of a model and equals the number of dimensions in which the query structure is outside the PD. VISDOM also displays the extent to which a compound is outside in each dimension. If a compound is outside in multiple dimensions, especially by a large extent (>2 standard deviations) in dimensions with high eigen values, the model is considered inapplicable to such a compound and prediction for such a compound may not be reliable.

2.2.2.4. Descriptors Outside the Training Domain (DescsOTD). This diagnostic simply counts the number of model descriptors whose values for the query compound are outside the range of the respective descriptors spanned by the training set. A large number of descriptors outside the training domain can be a sign of model extrapolation, especially when a significant portion of the structure is covered by these descriptors. VISDOM enables to visually map DescsOTD on the query structure individually or collectively to assess what portion of the structure is covered by such descriptors. The larger the structure covered by DescsOTD, the less reliable the prediction.

2.2.2.5. Number of New Two-Atom Fragments (NewFrags). This prediction diagnostic is a measure of new chemistry in the query molecule that is not represented in the training set. NewFrags is the number of two-atom fragments that were not present in the molecules in the training set but are present in the query structure. VISDOM users can visualize the extent of new chemistry in a query structure. The larger the structure unrepresented in the training set, the less reliable is the prediction, especially when the user has no extra knowledge about the effect of new chemistry on the property being predicted.

2.2.2.6. Model Coverage (ModCovr). This diagnostics measures coverage of the query structure by the descriptors in the predictive model. These are the descriptors that are common in the input molecule and the model, i. e., these are directly used in making prediction. The larger the ModCovr, the more reliable the prediction because these are the descriptors that have been determined during modeling to significantly impact the modeled property. VISDOM gives visual depiction of the coverage of query structure by the model descriptors.

2.2.2.7. QSAR Similarity Distance (SimDist). This diagnostic is a measure of similarity between query and the training set and equals the average Euclidian distance between the query structure and the five closest training set compounds. Similarity is measured using only the descriptors in the model. The smaller the SimDist, the higher the similarity of query to the training set. VISDOM lets users see each of the five closest compounds and model's performance on these compounds. When the model's predictions are accurate for highly similar compounds, the query's prediction can be accepted with higher confidence. However, if the compound closest to the query happens to be one of those identified as influential or outlier during model refinement, the confidence in the prediction may be lessened.

These diagnostics are checked prior to comparing model predictions with the observed values to objectively assess applicability of the model and reliability of a prediction. Ideally, for a prediction to be labeled "high-confidence", no diagnostic flags should be raised. However, it is likely that the prediction may not concord with the experimental value even when all prediction criteria are met because a model has inherent rates of specificity and sensitivity.

2.3. Molecular Design. In silico models are useful in making prediction of a property from chemical structure. However, another important application of in silico models is to benefit from the knowledge contained in them for guiding modulation of a structure toward a desired property. This requires back transformation of model's function and descriptors, called reverse QSAR (RQSAR) analysis.[24,25] By design, the MLR models of human iv CL and Vdss were developed to allow not only human PK prediction but also RQSAR analysis for chemical design in the early stages of discovery.

The RQSAR functionality, as implemented in VISDOM environment, gives quantitative contribution of each heavy atom, as embedded in the molecule, to the predicted value of a property. Any MLR QSAR model for property P in the VISDOM environment uses only E-state values on two-atom fragments as descriptors and, thus, can be expressed as

$$P = c + \sum w_i(\mathrm{E1}_i + \mathrm{E2}_i);$$

$$i = 1{-}p;\ \text{the number of descriptors}$$

where values of coefficients w have been obtained through regression analysis and E1 and E2 are the E-state values of the component atoms 1 and 2 in the two-atom fragment. Given these pieces of information and knowing in which descriptors a given atom is contributing, we can compute the total contribution of an atom to the predicted value of P.

Our RQSAR implementation, analogous to the moiety effect functionality in TOPKAT,[24] allows visual depiction of contribution from every atom and guides users to interactively

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Table 2. CL Predictions of 235 Test Compounds from 5 Random Splits of 525 Available Compounds

test set characteristic	no. cmpds	compounds predicted within fold deviation					
		50%	75%	90%	95%	99%	100%
all	235	1.75	2.67	4.77	7.25	16.1	1169.1
iv CL > 0.004	233[a]	1.73	2.63	4.45	6.89	10.83	16.1
predicted CL between 0.55 and 35	231	1.73	2.62	4.18	6.65	10.17	12.72

[a]7-hydroxystaurosporine (CAS no. 112953-11-4) was retained twice in test sets.

modulate the structure and assess which way a chosen property moves on making a change in the structure.

3. RESULTS AND DISCUSSION

3.1. Model Feasibility. For assessing the realistic predictive accuracy of in silico models of iv CL and Vdss models and to calibrate limitations of their applications, five random test and training sets comprising about 10% and 90%, respectively, of available compounds were created. The predictions obtained for each of the test sets from the corresponding training sets were compared with the data observed in the clinic. The accuracy of each model was assessed in terms of the distribution of fold deviations (FD), computed by eq 1, between predicted and the observed values:

$$\text{fold deviation} = \max(\text{PKe}, \text{PKp})/\min(\text{PKe}, \text{PKp}) \quad (1)$$

where PKe is the PK property observed in the clinic and PKp is that predicted by the model.

For iv CL, the random split of 525 available compounds led to the following number of training and test set compounds, respectively, in five sets: 475 and 50; 464 and 61; 487 and 38; 489 and 36; and 475 and 50. Since each split was independent of the other, one compound could be present in multiple test sets. However, each split was made on the data sorted on ascending values of iv CL (0.0037−24 mL/(min kg)) to ensure representation of almost complete range of CL values in both the training and test sets. Each training set was modeled as described in the Methods section, and the CL of the corresponding test set was predicted.

In all, we had 235 predictions. The quality of predictions is summarized (Table 2) in terms of fold deviations (FD) for a given percentage of compounds. For the entire test set of 235 compounds, 75% compounds are predicted with <3 FD—considered "good" prediction. However, the largest FD observed was over 1100. A closer examination revealed that this large deviation was for 7-hydroxystaurosporine (CAS no. 112953-11-4), present in two of the five test sets, with clinical iv CL of 0.0037 mL/(min kg). Although this compound was correctly predicted as having a very low CL, it was a single outlier in the distribution of fold deviations and was, thus, marked as a leverage point in the entire set. Therefore it should be noted that a limitation of the model is that it may not accurately predict CL of extremely low clearance compounds, an observation also recently made by Berellini et al.[26] After excluding 7-hydroxystaurosporine, all compounds are predicted within 16 FD and 95% compounds predicted within 7 FD. Further calibration of the model indicated that when the predicted CL was approximately between 2% and 200% of the hepatic blood flow (= 20 mL/(min kg)), the prediction was tighter than when prediction was outside this range.

This encouraging performance of in silico models in predicting clinical iv CL prompted us to develop a model from all 525 compounds and test it further with compounds not included in the training set.

3.2. SVR iv CL Model. The final SVR model for human iv CL was developed from all 525 compounds as described in the Methods section. Among the 77 fingerprints investigated, the one labeled "−CATSP13 −EC4:Y −RS −ecbig" returned the best average Q^2 of 0.32 from 50 cross-validation models; each created from randomly selected training (60%) and test (40%) compounds. This fingerprint incorporates structural information in terms of chemically advanced pharmacophores of up to 13 bonds (excluding hydrophobe−hydrophobe interactions), extended atom connectivity up to a four-bond radius (keeping all heavy halogens identical), and ring substitution patterns. We chose 40% for test sets versus leave one out (LOO) cross-validation because this gives a more realistic and conservative predictive accuracy. This may be one possible reason for somewhat low average Q^2 in cross-validation runs because LOO Q^2 is generally inflated. As mentioned by Golbraikh et al.,[27] a high value of LOO Q^2 is not the sufficient condition for the model to have a high predictive power but performance of the model on an external data set is the only way to establish a reliable QSAR model.

The final predictive model using all 525 compounds returns median fold deviation (eq 1) of 1.55 with 97.5% compounds fitted within 1.56-fold deviation. In spite of such tight fitting, here again, as observed in the feasibility studies above, for 7-hydroxystaurosporine (CAS no. 112953-11-4), a 44-fold deviation from the clinical CL of 0.0037 mL/(min kg) was observed. Although it was predicted by the model to be a low clearance compound (with predicted iv CL of 0.16 mL/(min kg)), we had expected a large deviation because the predicted value was below 2% of hepatic blood flow. The lack of fit of the final model for clinical CL of 0.0037 mL/(min kg), once again, indicated that the model may not accurately predict CL of extremely low clearance compounds, as observed by Berellini et al.[26] These feasibility studies led to a conclusion that the SVR in silico model is expected to return acceptable concordance with clinical studies for compounds with CL values between 0.5 and 35 mL/(min kg).

3.3. MLR iv CL Model. For the MLR model, the training set of 525 compounds led to a unique set of 653 two-atom fragments, whose E-state values were used as structure descriptors. These descriptors were subjected to objective selection and descriptors that were highly correlated (>0.9), redundant, or did not have nonzero value for at least three compounds were not considered in regression analysis. Preliminary model creation started with 358 descriptors, and a set of 91 potential descriptors (most with $p < 0.05$) was identified for further refinement of the model. During the refinement cycle of identifying influential/outlier[28,29]compounds and single descriptor-associated compounds, 2 descriptors were excluded and 15 compounds were set aside in the "outlier" set. Before accepting prediction for a

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Table 3. Comparison of Clinical and in silico Predicted iv CL for Test Sets from Yu[18] and Berellini et al.[26]

		predicted iv CL (mL/(min kg))		fold deviation		VISDOM diagnostics		
compound	clinical iv CL (mL/(min kg))	SVM	VISDOM	SVM	VISDOM	CL_Low	CL_Up	ApplIndx
acenocoumarol	1.28	1.028	4.048	1.2	3.2	2.961	5.535	0.056
actisomide	6.79	1.667	3.501	4.1	1.9	1.071	11.446	0.804
amifloxacin	4.56	2.729	3.106	1.7	1.5	2.388	4.040	0.040
apalcillin	1.9	2.891	2.134	1.5	1.1	1.470	3.096	0.079
bendamustine	4.49	2.541	3.017	1.8	1.5	1.368	6.653	0.358
bepridil	8.7	3.972	14.876	2.2	1.7	7.488	29.551	0.270
bidisomide	5.43	6.368	5.342	1.2	1.0	4.017	7.103	0.047
BLP1654	1.6	3.034	4.886	1.9	3.1	3.327	7.177	0.085
candoxatrilat	2.3	1.194	0.487	1.9	4.7	0.260	0.913	0.226
cefclidin	1.7	1.352	2.092	1.3	1.2	1.258	3.479	0.148
cefmenoxime	3.65	0.684	0.812	5.3	4.5	0.546	1.208	0.090
cefoselis	1.77	1.057	0.409	1.7	4.3	0.166	1.011	0.468
cefozopran	2.1	1.600	0.717	1.3	2.9	0.250	2.053	0.635
crizotinib[31]	9.6	6.520	3.789	1.5	2.5	2.120	6.771	0.193
dabigatran	1.93	4.140	2.418	2.1	1.3	1.534	3.812	0.119
deferasirox	0.84	1.355	2.832	1.6	3.4	2.042	3.925	0.061
diflomotecan	8.42	2.588	7.330	3.3	1.1	3.336	16.108	0.355
disufenton_sodium	1.35	0.675	0.754	2.0	1.8	0.308	1.842	0.458
doripenem	3.26	1.945	0.767	1.7	4.2	0.410	1.436	0.225
fenoprofen	0.6	1.213	3.887	2.0	6.5	2.659	5.682	0.083
fluphenazine	9.7	9.572	20.000	1.0	2.1	13.296	30.085	0.095
fulvestrant	11.8	4.315	10.355	2.7	1.1	4.718	22.728	0.354
garenoxacin	1.23	3.214	5.961	2.6	4.8	3.505	10.140	0.162
gavestinel	0.09	2.080	5.802	23.1	64.5	4.143	8.126	0.065
gliclazide	0.41	0.340	1.819	1.2	4.4	1.100	3.007	0.145
glufosfamide	1.6	2.606	0.052	1.6	30.7	0.005	0.592	3.386
glycopyrrolate	16.8	2.109	4.105	8.0	4.1	1.455	11.584	0.616
imidafenacin	6.1	2.333	5.501	2.6	1.1	4.002	7.563	0.058
iomeprol	1.4	2.742	9.854	2.0	7.0	5.058	19.195	0.255
isomazole	20.7	4.477	4.078	4.6	5.1	2.453	6.779	0.148
L-692429	3.05	2.163	5.195	1.4	1.7	2.744	9.835	0.233
levocabastine	0.43	2.218	0.342	5.2	1.3	0.108	1.082	0.761
levoprotiline	14.6	5.433	16.329	2.7	1.1	12.149	21.946	0.050
lubeluzole	1.7	9.016	13.070	5.3	7.7	6.016	28.397	0.345
maraviroc	9.4	5.152	123.491	1.8	13.1	42.143	361.865	0.662
mazapertine	6.17	6.194	8.595	1.0	1.4	6.162	11.988	0.063
metazosin	0.53	2.061	2.747	3.9	5.2	1.917	3.937	0.074
mianserin	21.9	6.902	17.956	3.2	1.2	5.977	53.943	0.693
patupilone	2.17	5.794	35.512	2.7	16.4	11.348	111.128	0.745
pelrinone	5.8	1.959	15.382	3.0	2.7	8.220	28.784	0.225
pidotimod	1.16	3.855	1.203	3.3	1.0	0.854	1.693	0.067
pirenzepine	2.77	3.311	3.192	1.2	1.2	2.500	4.075	0.034
practolol	2.28	6.124	7.646	2.7	3.4	6.046	9.670	0.032
propiverine	2.94	4.227	4.012	1.4	1.4	2.524	6.377	0.123
ridogrel	1.05	1.422	1.104	1.4	1.1	0.827	1.474	0.048
ritanserin	0.51	4.169	0.395	8.2	1.3	0.133	1.172	0.676
ritipenem	7.55	3.350	4.946	2.3	1.5	3.350	7.302	0.087
Ro25-6833	0.38	1.406	1.625	3.7	4.3	0.961	2.747	0.158
sabeluzole	4.05	9.750	15.518	2.4	3.8	7.468	32.244	0.306
semaxanib	14	3.443	30.037	4.1	2.1	11.957	75.457	0.486
sparfosic_acid	1.22	1.315	2.315	1.1	1.9	0.942	5.690	0.463
sulmazole	10	3.467	4.807	2.9	2.1	2.932	7.881	0.140
sulpiride	1.9	5.470	2.449	2.9	1.3	1.491	4.021	0.141
susalimod	0.07	0.632	0.475	9.0	6.8	0.318	0.708	0.091
tebufelone	8.9	3.281	22.915	2.7	2.6	5.555	94.521	1.150
treprostinil	10.7	3.828	10.753	2.8	1.0	7.458	15.504	0.077
vildagliptin	9.9	6.637	12.950	1.5	1.3	6.081	27.577	0.327

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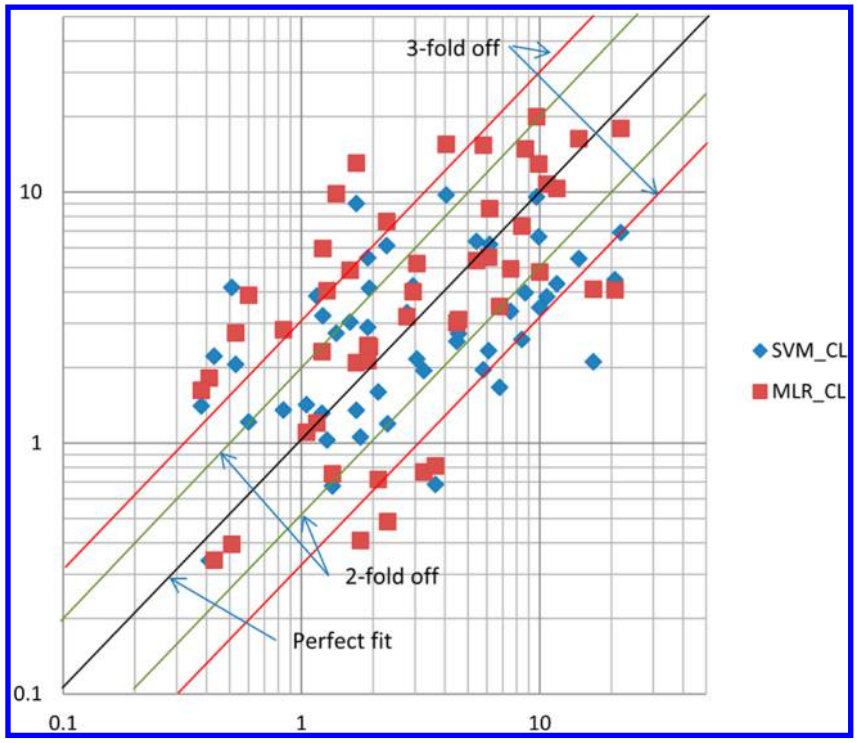

Figure 1. Log iv CL values observed in the clinic (x-axis) and predicted by the SVR and MLR in silico models. (black line) perfect fit; (green line) 2 FD; (red line) 3FD.

query structure, it is parsed against the outlier set to assess applicability of the model and reliability of its prediction.

The final 89-descriptor model from 510 compounds returned an R^2 of 0.70 and a degree-of-freedom-adjusted R_{adj}^2 of 0.64, indicating against unnecessary addition of descriptors (all descriptors are significant with absolute t-value of 1.73 or higher[30] and the largest condition index[28] being less than 30). The final MLR model was implemented in an in-house computational environment, VISDOM (visual in silico design of molecules), and its performance was evaluated on the same test sets as the SVR model.

3.4. iv CL Model Performance. To further test the performance of our iv CL predictors, the literature was scanned for compounds with human iv CL data not included in our training set of 525 compounds. The test data were collected from the Supporting Information provided in recent publications by Yu[18] and Berellini et al.[26] These were subjected to the same harmonization steps as the training set. The 56 compounds that survived these steps along with their clinical iv CL values and those predicted by the SVR and MLR models are collected in Table 3. The SVR and MLR models return median fold deviations of 2.2 and 2.1, respectively, and average fold deviations of 3.1 and 4.7. A closer examination revealed that for gavestinel (clinical iv CL of 0.086 mL/(min kg)) and susalimod (clinical iv CL of 0.07 mL/(min kg)) the SVR model gives large deviations of 23-fold and 9-fold and the MLR model returns 64.5 and 6.8 FD. As noted in the feasibility studies, both models predict low iv CL for these compounds, and therefore for compounds with extremely low clearance, the models may not return a tight fit. Exclusion of gavestinel and susalimod from the analysis reduces average fold deviation from SVR and MLR models to 2.6 and 3.6, respectively.

As mentioned in the Methods section, the implementation of the MLR model in the VISDOM environment allows examination of some prediction diagnostics to objectively assess reliability of a prediction. The composite prediction diagnostic, ApplNdx, along with the 95% confidence interval from the MLR model are also collected in Table 3. Clearly, the model is not applicable to glufosfamide and tebufelone because their AppNdx is above 0.8; the maximum value observed for the training set. Generally, the smaller the AppNdx value, the smaller is the likelihood of model extrapolation. However, one must evaluate all diagnostics mentioned in the Methods section collectively to assess reliability of a prediction. For instance, when confidence intervals are too wide, especially when other diagnostic flags are raised and the predicted value is much larger than the maximum value in the training set, one can expect a larger deviation between observed and predicted values. It can be seen that for maraviroc, patupilone, and semaxanib, the 95% confidence interval is fairly wide and the iv CL value predicted from the MLR model is much higher than the maximum value of CL in the training set. Therefore, for these compounds the MLR model is also deemed not applicable. Exclusion of these compounds from the accuracy analysis drops the average fold deviation from the MLR model to 2.6, comparable to that from the SVR model.

The agreement between the clinical iv CL values and those predicted by the SVR and MLR models for compounds to which either the MLR model is applicable or the observed value is not extremely small is shown in Figure 1. The mean, median, and maximum fold deviations from the MLR model for these 49 test compounds are, respectively, 2.64, 1.79, and 7.68 with 55% compounds being predicted with a 2 FD and 63% compounds being predicted within a 3 FD. On the other hand, the mean, median, and maximum fold deviations from the SVR model for these 49 test compounds are, respectively, 2.61, 2.15, and 8.17 with 45% of the compounds being predicted with a 2 FD and 76% compounds being predicted within a 3 FD.

The performance of the models was compared with the "assign average" (AA) model in which each compound was

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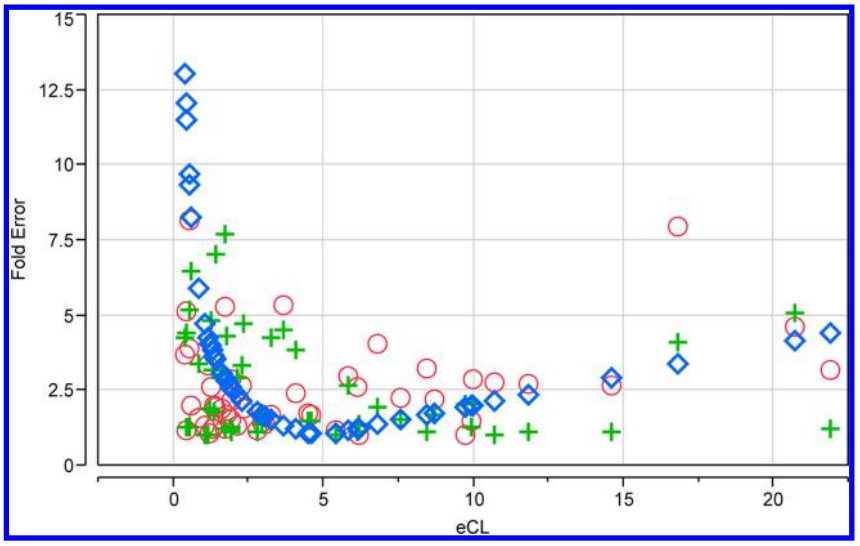

Figure 2. Distribution of fold errors from the SVR, MLR, and "AA" models for CL.

Table 4. Comparison of Clinical and in silico Predicted iv VDss for a Test Set from Berellini et al.[17]

		predicted VDss (L/kg)		fold deviation		VISDOM diagnostics		
CAS no.	clinical VDss (L/kg)	SVM	MLR	SVM	MLR	VD_Low	VD_Up	ApplNdx
103810-45-3	2.6	3.71	2.76	1.43	1.06	1.31	5.84	0.29
104383-17-7	5.5	4.98	6.82	1.11	1.24	1.97	23.68	0.81
110140-89-1	0.43	0.67	0.90	1.55	2.10	0.63	1.30	0.07
123122-54-3	0.25	0.19	0.05	1.30	5.53	0.03	0.07	0.12
134208-17-6	1.54	2.01	2.63	1.31	1.71	1.58	4.38	0.14
144665-07-6	2.6	5.00	9.42	1.92	3.62	2.75	32.23	0.79
145455-23-8	0.2	0.63	0.55	3.15	2.77	0.34	0.90	0.12
153436-22-7	0.12	0.26	0.19	2.15	1.58	0.12	0.30	0.12
201530-41-8	0.23	0.31	0.03	1.35	8.21	0.01	0.06	0.29
211914-51-1	0.85	0.82	0.52	1.04	1.65	0.26	1.01	0.24
274901-16-5	1.03	1.86	5.60	1.81	5.44	2.77	11.33	0.26
69-23-8	2.9	8.61	21.21	2.97	7.31	11.81	38.10	0.18
79516-68-0	1.17	0.44	0.34	2.63	3.47	0.20	0.58	0.15
81846-19-7	0.23	0.68	1.92	2.96	8.36	1.25	2.95	0.10
87051-43-2	1.41	4.19	8.15	2.97	5.78	2.86	23.19	0.57
96914-39-5	0.58	2.37	1.06	4.08	1.83	0.45	2.47	0.37
152-72-7	0.24	0.57	0.51	2.39	2.11	0.40	0.64	0.03
147-94-4	0.67	1.17	0.46	1.75	1.47	0.34	0.62	0.05
129453-61-8	4.15	0.90	325.23	4.59	78.37	67.28	1572.22	1.30
170105-16-5	1.51	0.99	2.14	1.52	1.42	1.29	3.55	0.14
94386-65-9	0.39	0.76	0.67	1.95	1.71	0.46	0.97	0.07
143248-63-9	0.68	1.42	0.83	2.08	1.22	0.68	1.00	0.02

assigned a predicted value equal to the average iv CL of the training set (= 5.08 mL/(min kg)). The mean, median, and maximum fold deviations obtained from the AA model are 6.24, 2.72, and 82.86. Although such a model has no mechanism of assessing prediction reliability, for a fair comparison we computed the mean, median, and maximum fold deviations obtained from the AA model for the 49 test set compounds mentioned above. These are 3.88, 2.76, and 15.26, which are significantly higher than those obtained by either the SVR or the MLR model. It was observed that the AA model predicts only 41% compounds within 2 FD and 53% compounds within 3 FD. Furthermore, the deviations from the AA model are not randomly distributed (Figure 2); higher deviations will result at extreme ends of CL values and tighter predictions will naturally result for CL values around 5 mL/(min kg). This analysis indicates that the AA model is not a meaningful option.

3.5. iv Vdss Models. Although the data set for Vdss models was different (569 compounds vs 525 compounds for CL), the same approach employed for CL was followed to establish feasibility of a predictive Vdss model. Here we describe only the final models derived from the total training set. As in the case of the iv CL models, it was observed during the feasibility studies that the model is likely to make poor prediction for compounds with very low Vdss (<0.1 L/kg). The predictive accuracy of the Vdss models was evaluated on two external sets retrieved from the literature.[32]

3.5.1. SVR iv Vdss Model. The fingerprint "−CATS −ECC3 −CLOGP", among the 77 fingerprints investigated, returned the best average Q^2 of 0.55 from 50 cross-validation models;

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Table 5. Comparison of Clinical and in silico Predicted VDss for a Test Set from Poulin and Theil[32]

compound	clinical VDss (L/kg)	predicted VDss (L/kg)				
		MLR	95%_Low	95%_High	SVM	Poulin[32]
ascorbic acid	0.72	0.187	0.065	0.534	0.525	0.5
cloprendol	0.81 ± 0.14	0.776	0.478	1.259	0.940	0.88
coumarin	1.29	1.026	0.845	1.246	1.271	1.08
cyctosine_arabinoside	0.44 to 0.86	0.456	0.338	0.616	1.169	0.55
dideoxyinosine	0.73 to 0.77	2.294	1.466	3.592	1.259	0.55
epiroprim	2.62 ± 0.36	2.110	1.388	3.208	1.671	2.81
etidocaine	1.15	1.415	0.974	2.057	1.180	3.31
griseofulvin	1.4	0.775	0.520	1.157	0.740	1.72
sulpride	0.849 ± 0.116	0.326	0.162	0.656	1.762	0.64

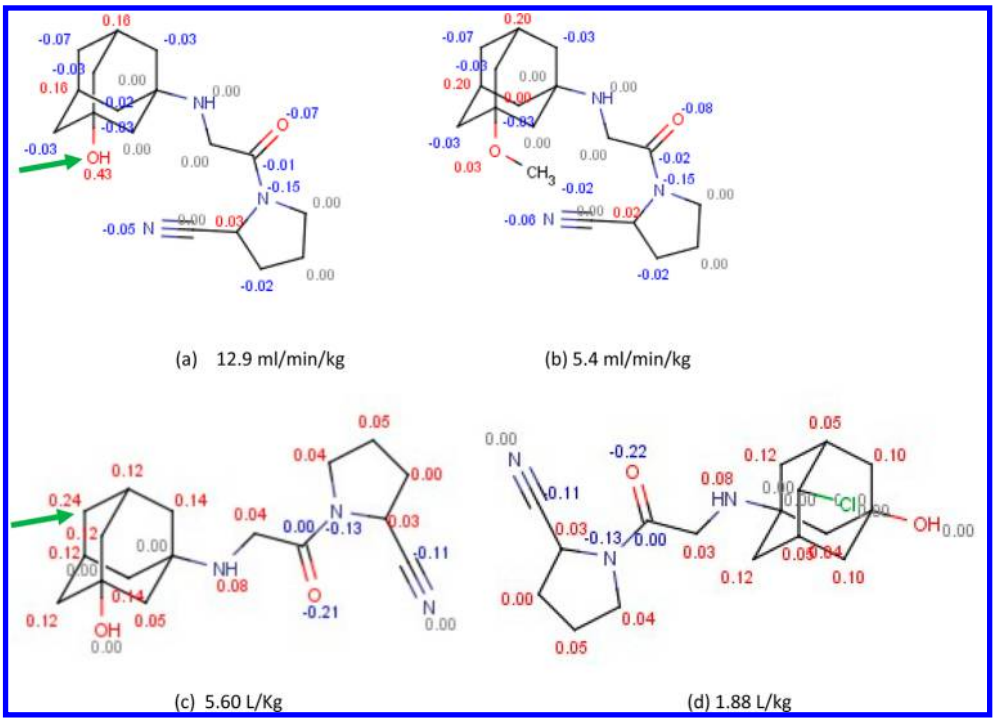

Figure 3. Contributions of atom-types by RQSAR Analysis: (a) iv CL of vildagliptin, (b) iv CL of O-methylated vildagliptin, (c) iv Vdss of vildagliptin, (d) iv Vdss of chlorinated vildagliptin.

each created from randomly selected training (60%) and test (40%) compounds. This fingerprint incorporates pharmacophore information without hydrophobe−hydrophobe interaction, extended atom connectivity up to a three-bond radius (keeping all heavy halogens identical), and binned cLogP. The final predictive model, built with this fingerprint using all 569 compounds, returns median fold deviation (eq 1) of 1.62 with 97.5% compounds fitted within 1.95-fold deviation. The best-fitting model shows the largest observed deviation of 8.86 fold for hydroxychloroquine (CAS no. 118-42-3).

3.5.2. MLR iv Vdss Model. For the MLR model, the training set of 569 compounds led to a unique set of 660 two-atom fragments, whose E-state values were used as structure descriptors. After subjecting these descriptors to the objective feature selection steps, identical to those followed for building the CL model, 365 descriptors remained for initial model development. The preliminary model with 91 potential descriptors (most with $p < 0.05$) was finally refined to an 85-descriptor model and 9 influential/outlier[28,29] compounds were identified and set aside. Before accepting its prediction, a query structure is parsed against the outlier set to assess applicability of the model and reliability of the prediction.

The final 85-descriptor model from 560 compounds returned an R^2 of 0.782 and a degree-of-freedom-adjusted R_{adj}^2 of 0.743, indicating against unnecessary addition of descriptors (all descriptors are significant with absolute t-value of 1.73 or higher[30] and the largest condition index[28] being less than 30).

3.6. Vdss Model Performance. One of the two test sets we used to check the performance of our Vdss predictors on compounds not included in our training set was taken from Berellini et al.[17] This set had 29 compounds with human Vdss data but five of these failed our Discovery Space filter and definite structures could not be retrieved for two additional compounds (CAS nos. 532959-63-0 and 220246-81-1). A comparison of the clinical and predicted Vdss values for the remaining 22 compounds is given in Table 4. The SVR model gives an average deviation of 2.2 fold with the largest deviation of 4.6 fold for Fulvestrant (CAS no. 129453-61-8). Here, 82% compounds are predicted within 3 FD, which is in line with the prediction accuracy reported by Berellini et al.[17]

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For the MLR model in the VISDOM environment, the prediction diagnostics were examined to assess reliability of predictions before making a comparison between predicted and clinical iv Vdss values. In Table 4, from the large value (= 1.3) of the reliability diagnostic ApplNdx for fulvestrant (CAS no. 129453-61-8), it is clear that the MLR model is not applicable to this compound. A deviation of 78.4-fold was noted for this compound. Similarly, compounds with wide 95% confidence intervals, especially when the predicted value is large, are generally expected to give larger deviation. This can be seen for compounds with CAS no. 69-23-8 (7.31 FD), 87051-43-2 (5.78 FD), and 144665-07-6 (3.62 FD). The model was deemed applicable to the remaining compounds giving 3.04 FD on an average. A closer look revealed 123122-54-3 and 201530-41-8 were not predicted well ((5.53 and 8.21 FD, respectively). As seen in the feasibility studies, the model was likely to produce large FD values for these two compounds because they have very low predicted Vdss values; 0.05 and 0.03 L/kg, respectively. It is quite clear that application of prediction diagnostics and application of calibrations from feasibility studies can help determine the reliability of predictions.

Another test set to evaluate the performance of iv Vdss models was taken from Poulin and Theil.[32] The reported values are compared with the Vdss values predicted from our complete in silico model and from the mechanism-based model proposed by Poulin and Theil in Table 5. It can be seen that in silico SVR model performs as well as the Poulin and Theil's method which requires pK_a, fup, and LogD data of a compound as input.

For the Poulin and Theil[32] set, no diagnostic flags were raised by the MLR model installed in VISDOM. For all compounds exceptdideoxyinosine, the values reported from the clinic are within the 95% confidence interval predicted by our model. It can be seen that, like the SVR model, our iv Vdss MLR in silico model requiring just the 2D structure input gives as good an agreement with the clinical Vdss data as well as those from the Poulin and Theil's method which requires pK_a, fup, and LogD data of a compound as input.

3.7. Model Application in Molecular Design. Both SVR and MLR models are useful in prediction of human iv CL and Vdss, the MLR models installed in the VISDOM environment can be subjected to reverse QSAR (RQSAR) analysis to get a quantitative contribution of each atom to the predicted value and help model-guided modulation of structures in silico for moving CL and Vdss in the desired direction. As an example, the results of RQSAR analysis of vildagliptin (274901-16-5) along with the contributions of atoms to the predicted value of human iv CL are shown in Figure 3a. The interpretation here is that the atoms, as embedded in the molecule, associated with positive contributions are likely to increase iv CL and those associated with negative numbers will tend to reduce the iv CL. That is, the OH group in this molecule has the largest positive contribution. To modulate this structure toward lower iv CL, this OH could be a good starting point. Since each shown contribution is that of the atom as embedded in the molecule, one does not necessarily need to drop OH from the molecule; methylation of the O, for example, will effectively remove OH. We replaced the OH with the OCH3 and applied the RQSAR tool. The contributions of atoms to the predicted iv CL of the O-methylated modification are also shown in Figure 3b. The predicted value for the O-methylated modified structure is 5.4 mL/(min kg), lower than 12.9 mL/(min kg) of the original molecule, vildagliptin.

The RQSAR analysis of vildagliptin for iv Vdss is depicted in Figure 3c. At the outset, it can be seen that one atom type can have different impact in modulating different properties. The impact of the −OH group on iv CL is +0.43, but its contribution to iv Vdss is 0.00; thus, according to our MLR models for iv CL and iv Vdss, the −OH group leads to increase in iv CL but it is innocuous for iv Vdss. On the other hand, the cyclic methylene group marked by green arrows has a negative contribution to iv CL but a positive contribution to iv Vdss. The predicted iv Vdss of vildagliptin is 5.60 L/kg. A modification of the marked methylene group by chlorination leads to a predicted iv Vdss of 1.88 L/kg because an atom type with positive contribution (= 0.24) no longer exists in the molecule. The contributions of various atoms to iv Vdss of chlorinated vildagliptin are shown in Figure 3d.

4. CONCLUSIONS

Predicting pharmacokinetics (PK) in humans for new chemical entities is essential for appropriately predicting dose and dosing regimen. Traditional experimental approaches to assess important PK parameters CL and Vdss in humans involve extrapolation of multiple preclinical PK measurements to humans, and are, thus, expensive and time-consuming. This paper has demonstrated that predictive in silico models of human iv CL and Vdss are feasible. These models are based entirely on structural descriptors and therefore avoid interspecies extrapolation. Incorporation of RQSAR algorithm, along with a robust, predictive in silico model can be a valuable design tool in optimizing cost-intensive properties early in the discovery process. It is very important to understand the scope of application of a model and to assess reliability of a prediction, especially, with linear modeling methods, before accepting a prediction to analyze model performance. For instance, the SVR and MLR models presented here are likely to make poor prediction for compounds with very low CL (<0.5 mL/(min kg)) or very high CL (>24 mL/(min kg)) and very low Vdss (<0.1 L/kg); although categorical classification remains viable at theses extremes.

■ ASSOCIATED CONTENT

Supporting Information

Data sets for modeling Vdss and CL. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PK, phramacokinetics; CL, clearance; Vd, volume of distribution; Vdss, steady state volume of distribution; ADME, absorption distribution, metabolism, excretion; QSAR, quantitative structure−activity relationship; RQSAR, reverse quantitative structure activity relationship; LOO, leave-one-out; SVR, support vector regression; MLR, multiple linear regression;

DSF, Discovery Space filter; CAS, Chemical Abstract Service; VISDOM, visual in silico design of molecules; FD, fold deviation

REFERENCES

(1) Wang, J.; Urban, L. The impact of early ADME profiling on drug discovery and development strategy. *Drug Discovery World.* **2004**, 73−86.

(2) Tang, H.; Hussain, A.; Leal, M.; Mayershon, M.; Fluhler, E. Interspecies Prediction of Human Drug Clearance Based on Scaling Data from One or Two Animal Species. *Drug Metab. Dispos.* **2007**, *35*, 1886−1893.

(3) 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.

(4) Ito, K.; Houston, B. Prediction of Human Drug Clearance from *in Vitro* and Preclinical data Using Physiologically Based and Empirical Approaches. *Pharm. Res.* **2005**, *22*, 103−112.

(5) 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. Exptl. Therapeut.* **1997**, *283*, 46−58.

(6) Hosea, N. A.; Collard, W. T.; Cole, S.; Maurer, T. S.; Fang, R. X.; Jones, H.; Kakar, S. M.; Nakai, Y.; Smith, B. J.; Webster, R.; Beaumont, K. Prediction of Human Pharmacokinetics From Preclinical Information: Comparative Accuracy of Quantitative Prediction Approaches. *J. Clin. Pharmacol.* **2009**, *49* (5), 513−533.

(7) Norinder, U.; Bergstroem, C. A. S. Prediction of ADMET Properties. *Chem. Biol.* **2007**, *3*, 1003−1042.

(8) Gombar, V. K.; Silver, I. S.; Zhao, Z. Role of ADME Characteristics in Drug Discovery and Their *In Silico* Evaluation: *In Silico* Screening of Chemicals for Their Metabolic Stability. *Curr. Topics Med. Chem.* **2000**, *3* (11), 1205−1225.

(9) Derety, E.; Feher, M.; Schmidt, J. M. Rapid Prediction of Human Intestinal Absorption. *Quant. Struct.-Act. Relat.* **2002**, *21*, 493−506.

(10) Manga, N.; Duffy, J. C.; Rowe, P. H.; Cronin, M. T. D. A Hierarchical QSAR Model for Urinary Excretion of Drugs in Humans as a Predictive Tool for Biotransformation. *Quant. Struct.-Act. Relat.* **2003**, *22*, 263−273.

(11) Norinder, U.; Haeberlein, M. Computational Approaches to the prediction of the Blood-Brain Distribution. *Adv. Drug Delivery Rev.* **2004**, *54*, 291−313.

(12) Wajima, T.; Fukumara, 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.

(13) Turner, J. V.; Maddalena, D. J.; Agatonovic-Kustrin, S. Bioavailability Prediction Based on Molecular Structure for a Diverse Series of Drugs. *Pharm. Res.* **2004**, *21*, 68−82.

(14) Yoshida, F.; Topliss, J. G. QSAR Model for Drug Human Oral Bioavailability. *J. Med. Chem.* **2000**, *43*, 2575−2585.

(15) Andrews, C. W.; Bennett, L.; Yu, L. X. Predicting Human Oral Bioavailability of a Compound: Development of a Novel Quantitative Structure-Bioavailability Relationship. *Pharm. Res.* **2000**, *17*, 639−644.

(16) Moda, T. L.; Montanari, C. A.; Andricopulo, A. D. Hologram QSAR Model of the Prediction of Human Oral Bioavailability. *Bioorgan. Med. Chem.* **2007**, *15*, 7738−7745.

(17) Berellini, G.; Springer, C.; Waters, N. J.; Lombardo, F. *In silico* Prediction of Volume of Distribution in Human Using Linear and Nonlinear Models on a 669 Compound Data Set. *J. Med. Chem.* **2009**, *52*, 4488−4495.

(18) Yu, M. J. Predicting Total Clearance in Humans from Chemical Structure. *J. Chem. Inf. Model.* **2010**, *50*, 1284−1295.

(19) Obach, R. S.; Lombardo, F.; Waters, N. J. Trend Analysis of a Database of Intravenous Pharamacokinetic Parameters in Humans for 670 Drug Compounds. *Drug Metab. Dispos.* **2008**, *36*, 1385−1405.

(20) Ivanciuc, O. Applications of Support Vector Machines in Chemistry. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Cundari, T. R., Eds.; Wiley-VCH: Weinheim, 2007; Vol *23*, pp 291−400.

(21) Kier, L. B.; Hall, H. *Molecular Structure description: The Electrotopological State.*; Academic Press: San Diego, CA, 1999.

(22) Mandel, J. The Regression Analysis of Collinear Data. *J. Res. Natnl. Bur. Stand.* **1985**, *90* (6), 465−478.

(23) Mandel, J. Use of the Singular Value Decomposition in Regression Analysis. *Am. Stat.* **1982**, *36*, 15−24.

(24) Ruiz, P.; Mumtaz, M.; Gombar, V. Assessing the toxic effects of ethylene glycol ethers using Quantitative Structure Toxicity Relationship models. *Toxicol. Appl. Pharmacol.* **2011**, *254*, 198−205.

(25) Martin, S. Lattice Enumeration for Inverse Molecular Design Using the Signature Descriptor. *J. Chem. Inf. Model.* **2012**, *52*, 1787−1797.

(26) Berellini, G.; Waters, N. J.; Lombardo, F. In Silico Prediction of Total Human Plasma Clearance. *J. Chem. Inf. Model.* **2012**, *52*, 2069−2078.

(27) Golbraikh, A.; Tropsha, A. Beware of q^2. *J Mol Graph Model.* **2002**, *20* (4), 269−276.

(28) Rousseeuw, P. J.; Leroy, A. M. *Robust Regression & Outlier Detection*; John Wiley & Sons: New York, 1987.

(29) Cook, R. D. Detection of influential observations in linear regression. *Technometrics* **1977**, *19*, 15−18.

(30) Costanza, M. C.; Afifi, A. A. Comparison of stopping rules in forward stepwise discriminant analysis. *J. Am. Statist. Assoc.* **1979**, *74*, 777−785.

(31) Center for Drug Evaluation and Research. *Clinical Pharmacology and Biopharmaceutics Review(s)*; March 30, 2011; Application Number: 202570Orig1s000.

(32) Poulin, P.; Theil, F.-P. Prediction of Pharmacokinetics Prior to *In Vivo* Studies. 1. Mechanism-Based Prediction of Volume of Distribution. *J. Pharm. Sci.* **2002**, *91*, 129−156.