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BDDCS Class Prediction for New Molecular Entities

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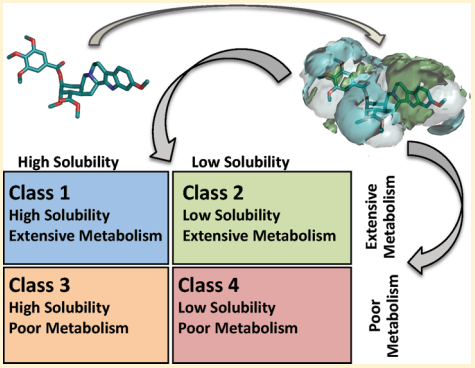
Supporting Information

ABSTRACT: The Biopharmaceutics Drug Disposition Classification System (BDDCS) was successfully employed for predicting drug−drug interactions (DDIs) with respect to drug metabolizing enzymes (DMEs), drug transporters and their interplay. The major assumption of BDDCS is that the extent of metabolism (EoM) predicts high versus low intestinal permeability rate, and *vice versa*, at least when uptake transporters or paracellular transport is not involved. We recently published a collection of over 900 marketed drugs classified for BDDCS. We suggest that a reliable model for predicting BDDCS class, integrated with *in vitro* assays, could anticipate disposition and potential DDIs of new molecular entities (NMEs). Here we describe a computational procedure for predicting BDDCS class from molecular structures. The model was trained on a set of 300 oral drugs, and validated on an external set of 379 oral drugs, using 17 descriptors calculated or derived from the VolSurf+ software. For each molecule, a probability of BDDCS class membership was given, based on predicted EoM, FDA solubility (FDAS) and their confidence scores. The accuracy in predicting FDAS was 78% in training and 77% in validation, while for EoM prediction the accuracy was 82% in training and 79% in external validation. The actual BDDCS class corresponded to the highest ranked calculated class for 55% of the validation molecules, and it was within the top two ranked more than 92% of the time. The unbalanced stratification of the data set did not affect the prediction, which showed highest accuracy in predicting classes 2 and 3 with respect to the most populated class 1. For class 4 drugs a general lack of predictability was observed. A linear discriminant analysis (LDA) confirming the degree of accuracy for the prediction of the different BDDCS classes is tied to the structure of the data set. This model could routinely be used in early drug discovery to prioritize *in vitro* tests for NMEs (e.g., affinity to transporters, intestinal metabolism, intestinal absorption and plasma protein binding). We further applied the BDDCS prediction model on a large set of medicinal chemistry compounds (over 30,000 chemicals). Based on this application, we suggest that solubility, and not permeability, is the major difference between NMEs and drugs. We anticipate that the forecast of BDDCS categories in early drug discovery may lead to a significant R&D cost reduction.



KEYWORDS: *BDDCS, ADMET, GRID, MIF, drug disposition, drug−drug interactions, VolSurf+, FDA solubility, machine learning*

INTRODUCTION

In the past decade, the US Food and Drug Administration (FDA) has adopted the Biopharmaceutics Classification System (BCS) as a criterion for biowaivers: for highly permeable and soluble drugs, admission onto the market of immediate release oral products of those drugs was facilitated by a waiver for *in vivo* bioequivalence studies.[1,2] When introducing the BDDCS, Wu and Benet recognized a strong correlation between EoM and intestinal permeability rate.[3] The EoM should be adopted[4] as a surrogate for intestinal permeability, allowing extensively metabolized and highly soluble BDDCS class 1 drugs to be eligible for biowaivers. This scheme was also adopted by the European Medicines Agency (EMA).[5]

More recently BDDCS was successfully employed for rationalizing DDIs with respect to metabolism alteration, transporter modulation and metabolizing enzyme−transporter interplay in the gut and in the liver.[6] By definition the BDDCS scheme provides an estimation of the potential impact of DME inhibition (or induction); that is, DME inhibitors are expected not to affect the disposition of drugs that are poorly metabolized *in vivo*. In addition, BDDCS can be used as a

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tool to predict the effect arising from the coadministration with a modulator of transporter activity in the gut and in the liver. The expected behavior for drugs classified according to BDDCS in the gut can be summarized as follows: class 1 compounds are both highly soluble in the gut and highly permeable, therefore their intestinal permeability and fraction absorbed (Fa) are not significantly affected by transporters, possibly due to saturation. Thus, if a class 1 drug is an *in vitro* substrate for a transporter expressed in gut, inhibition or induction of that transporter will not have any clinically relevant *in vivo* effect on intestinal absorption or metabolism. Class 2 drugs are highly permeable, thus their Fa is not significantly affected by transporters. However, due to their comparatively lower water solubility, class 2 drugs are unlikely to saturate efflux transporters in the gut, therefore inhibiting efflux transporters may lead to altered exposure to DMEs in the gut, higher fraction nonmetabolized in the gut (Fg) and higher plasma concentration.[7,8] The inhibition of intestinal uptake transporters is expected to be not relevant for this class. For class 3 and class 4 drugs, the intestinal permeability is strongly affected by both uptake and efflux transporters: these drugs require active transport to overcome their poor passive permeability. The inhibition or the induction of any intestinal transporter has a potential to cause clinically relevant changes in the disposition of poorly metabolized drugs. A major substantial difference between BDDCS and BCS is that highly soluble, poorly metabolized drugs (BDDCS class 3) could be BCS class 1 when their absorption is mediated by uptake transporters or paracellular passage. Thus, BCS is less accurate in predicting DDIs. Use of BDDCS in predicting DDIs in the liver has been extensively addressed elsewhere, and it is beyond the aim of this work.[6]

The fraction of drugs with undesirable ADME properties that reach clinical trials is no longer a major issue for industrial R&D;[9] more critical now are early phase toxicity optimization and clinical efficacy. The ability to predict BDDCS categories could serve to better anticipate DDIs and other limitations related to drug disposition, and could help prioritize the sequence of *in vitro* assays. Thus, *in vitro* testing could focus on those NMEs that are potentially substrates for transporters *in vivo*. For example, class 2 NMEs should be tested for efflux transporters only if they are substrates for intestinal phase 1 enzymes (predominantly CYP3A, but also CYP1A2, with CYP2C9 and CYP2C19 less likely) or intestinal phase 2 enzymes (predominantly glucuronosyl- and sulfo-tranferases), or if their potential clinical use is in the CNS area. Class 3 and 4 NMEs should be first tested for uptake transporters and then for efflux transporters. NMEs predicted as class 1 could be eligible for several "*in vitro* waivers" in the early phases. Thus, we anticipate that the forecast of BDDCS categories in early drug discovery may lead to a significant cost reduction.

In our recent compilation[10] of BDDCS classification for over 900 drugs, we provided some analytical discussion of the distribution of calculated properties. We suggested that either measured or calculated LogP could be confidently related to the EoM for drugs only outside the LogP interval from 0 to 2. Takagi et al.[11] observed that cLogP correctly predicts high versus low permeability 2/3 of the time. This could be explained by the link between passive transcellular permeability and EoM, and the degree of lipophilicity of the drug in question.[12] This relationship is likely to explain the large overlap between BCS and BDDCS assignment. A corollary would be that the correlation fails when drug permeability is mainly determined by active uptake or paracellular passage; these theoretical assumptions are currently being investigated by the Benet lab.

We further pointed out[10] that the lowest calculated VolSurf+ solubility[13,14] in the pH range between 3 and 7.5 is a good predictor for FDAS when HDS is known, at least for BDDCS classes 1, 2, and 3. FDAS is defined based on dose number (DN), an estimate of the ability of the drug at its HDS to completely dissolve in 250 mL of water. The solubility considered to calculate the DN is the lowest over a pH range between 1 and 7.5 at 37 °C. Prediction failed for class 4 drugs probably due to self-association in water.[10,15] FDAS is a property of the drug in a formulation and is not an intrinsic property of the active pharmaceutical ingredient (API) itself. Indeed, HDS cannot be defined for NMEs in the absence of a clinical context. Recognizing this major limitation, we hereby propose a model based on the two properties that define the BDDCS framework: one estimator is used for FDAS, and one for EoM. The prediction outcome consists of 4 different probability scores, which combine to estimate the membership of each BDDCS class. The model is trained by a set of 300 oral drugs and validated by a set of 369 oral drugs. Performances of the model and validity of the strategy adopted are evaluated by data mining and comparison with earlier BDDCS modeling efforts.

The model presented in this work was built using descriptors directly or indirectly calculated from VolSurf+. The VolSurf+ software calculates molecular descriptors based on the three-dimensional (3D) GRID[16] molecular interaction fields (MIFs). This software has been successfully utilized to predict ADME Tox properties for drugs in a number of published scientific papers.[13,14,17−22] We also used data mining to derive a small number of highly informative descriptors. This low dimensional matrix was employed to create three models for each property (FDAS and EoM), models that were used as "votes" in two consensus models. The final BDDCS probability estimate was determined using the predictions of the consensus models, as well as their degree of agreement.

The model performance is discussed in detail, as we consider its possible use in early drug discovery. Furthermore, we evaluated the feasibility of performing BDDCS predictions for a large medicinal chemistry data set.[23] This data set contains NMEs that are a typical sample for what might be encountered in early drug discovery and is used to illustrate the distribution of BDDCS classes among NMEs.

EXPERIMENTAL SECTION

Data Set. The data set was extracted from our earlier BDDCS compilation.[10] From the over 900 drugs that we have annotated, we selected 697 orally formulated drugs, from which we further selected those that have their HDS expressed as a mass quantity, not as a concentration (e.g., excluding solutions or suspensions). For the remaining 693 drugs, we calculated the dose number (DN) as follows (eq 1):

$$\mathrm{DN} = (\mathrm{HDS}/250\,\mathrm{mL})/\mathrm{Cs} \quad (1)$$

where Cs is the *minimum* solubility in the pH range from 1 to 7.5 at 37 °C. Using this criterion, if DN is above 1, it corresponds to low FDAS, while DN ≤ 1 corresponds to high FDAS. When numerical values were not available for "practically insoluble" drugs, the standard value of 0.01 mg/mL was adopted as suggested by Takagi et al.[11] Calcitrol, cabergoline, dutasteride, proscillaridin, etizolam, pergolide,

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vitamin D2, repaglinide, sirolimus, paricalcitol and tibolone were "practically insoluble" drugs having HDS equal to or below 2.5 mg, thus DN was equal to or below 1. Because 0.01 is an arbitrary value and because lower solubility is likely for these drugs, they were excluded. Due to lack of parametrization in VolSurf+, we also excluded inorganic salts (ferrous sulfate, potassium chloride) and auranofin. This left a total of 679 drugs that composed the final set. Since multiple modeling strategies were explored, the exact composition of the training and the validation sets differed for each approach.

Molecular Descriptor Calculation. A number of 128 VolSurf+ descriptors were calculated importing each molecule at pH 7.5 and using default options (see Table S1 in the Supporting Information). In addition, a number of solubility descriptors were adapted from VolSurf+ for BDDCS prediction: minVSLgS3-7.5, the minimum calculated solubility in the pH range between 3 and 7.5; and the predicted dose numbers (PDN) at 3, 10, 75, 209, 250, 500 mg. These are hypothetical doses that correspond to the 10th percentile, the 25th percentile, the median, the average, the 75th percentile and 90th percentile of the HDS distribution for our entire data set.

For the different hypothetical doses, PDN was derived as follows (eq 2):

$$\text{PDN-HD} = (\text{HD}/250)/(10^{\text{minVSLgS3-7.5}} \times \text{MW}) \qquad (2)$$

where HD is the hypothetical dose and MW is the molecular weight as calculated by VolSurf+. The predicted dose number at marketed HDS (PDN-MRKT) was introduced in the data matrix only to evaluate the influence of knowing HDS on the accuracy of BDDCS prediction.

Modeling Strategy and Feature Selection. Data were analyzed and processed using the data mining software Orange Canvas,[24] the R package MASS,[25] and the cheminformatics web tool Chembench.[26,27] Earlier BDDCS models[17] predicted only 1 class out of 4 with an accuracy above 50%, indicating that a single four-label classification model is likely to be biased toward the most populated class. We therefore proceeded to verify that the one-class bias is due to the modeling strategy using the Chembench implementation of the support vector machine[28] (SVM) and random forest[29] (RF) algorithms by performing 2 four-label classification models. For these models the training set was composed of 80% of each BDDCS class, randomly selected via Chembench. The SVM model was 64% accurate in external predictions, an improvement with respect to the earlier models (Table S20 in the Supporting Information).[17] However, we found no significant improvement in terms of average BDDCS class accuracy (44%). To further confirm the intrinsic limitations of this data set we also performed a LDA analysis inclusive of 679 drugs (Figure 1). Class 2 drugs were easily separated from class 3 drugs, and *vice versa*. However these two classes partially overlap with class 1. Class 4 drugs could not be discriminated from the drugs in any other class (Table S20 in the Supporting Information).

Given the above, we opted for independent, separate predictions for FDAS and EoM in order to maintain individual ranking for the different BDDCS classes, a strategy that is further supported by the fact that BDDCS itself is a two-parameter (EoM, FDAS) classification scheme. We trained the FDAS model using 100 class 1 and 100 class 2 drugs, whereas the EoM model was trained using 100 class 1 and 100 class 3 drugs. The choice of training set is based on the distribution of

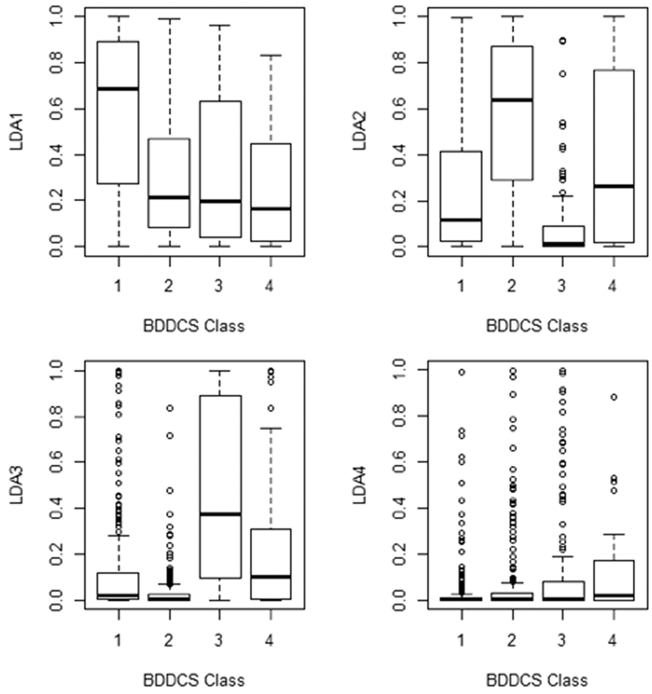

Figure 1. Box plots showing the distribution of BDDCS classes in the linear discriminant space. Boxes delimit values between the 25th and the 75th percentile, the line contained in the box is the median, whiskers correspond to the maximum and the minimum values, and the dots are outliers.

FDAS- and EoM-related properties. Within the framework of the statistical tools and descriptors used here, we could not identify features that can distinguish BDDCS class 4 from the others. To avoid the inclusion of confounding information into the training set, this class was therefore used only in external validation. For both the FDAS and the EoM models the external validation set consisted of 479 drugs, although the exact composition of the test sets was not identical. Orange Canvas allows the user to evaluate the impact of each variable on the entropy of a data set in terms of information gain.[24] Thus, when modeling EoM and FDAS, we considered only the top 30 descriptors, as ranked by information gain. An exhaustive search for the optimal number and combination of descriptors was performed using the naive Bayes and kNN (k nearest neighbors) classifiers. The selection was done iteratively by adding descriptors in their decreasing rank of information gain, and keeping them in the model only when the classification model accuracy increased, as judged by 5-fold cross-validation. For each newly introduced descriptor, the other descriptors were removed one by one (leave-one-out) in order to maintain model simplicity, keep its dimensionality low, and preserve accuracy. For each new (sub)optimal descriptor combination, the iterative process was restarted using the highest informative and the unselected descriptors. The method is generally reproducible for naive Bayes, but not fully for kNN, since there is the arbitrary choice of the initial k number of neighbors. The descriptors selected by the two models were employed to create other models with other classifiers, such as SVM using a different kind of kernel functions. For both FDAS and EoM, the 3 best models in external validation were used as "votes" in a consensus model. The final estimate for each model was determined by the "majority wins" logic; however, the degree of agreement in predictions was translated into a

confidence value for each prediction. The performance of every model is available in the Supporting Information, together with the parameters and the descriptors used (Table S3 in the Supporting Information). The final BDDCS class prediction is given based on calculated FDAS and EoM values, as well as their confidence scores (Table S4 in the Supporting Information).

The probability of membership in each BDDCS class can be also used as a model to predict one class versus the others via two-label models. Chembench was used to compute two-class models for each BDDCS class for comparison purpose. Two-class models derived from Chembench were trained by the 80% of the molecules belonging to the target class and the 80% of the remaining molecules. The most performing of the RF and the SVM models are reported for each BDDCS class (Table S20 in the Supporting Information).

RESULTS

FDA Solubility Prediction. The three best models for FDAS prediction were (i) a naive Bayes model using 4 variables, with classification accuracy of 78% for the training set and 76% during external validation; (ii) a kNN model using 6 variables and classification accuracy of 80% for training and 74% for the external validation set; and (iii) a SVM (linear kernel) using 9 variables, and classification accuracy of 75% for both training and validation set (see Tables S3 and S4 in the Supporting Information). The resulting consensus model had an accuracy of classification of 78% for the training set and 77% for the external validation set, respectively (Figure 2). The external prediction accuracy for the single BDDCS classes was, in decreasing order of accuracy, as follows: class 3, class 2, class 1 and class 4. Notably, even when class 3 drugs were excluded from the training set, they remained the most accurately predicted class, confirming the validity of our strategy.

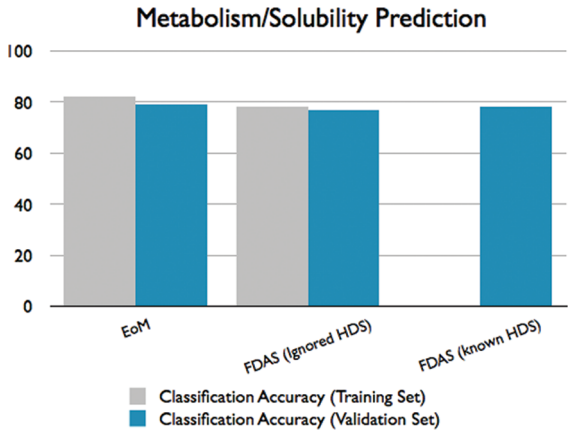

Figure 2. Classification accuracy for EoM and FDA solubility predictions. Accuracy in predicting FDAS is presented for (i) a model that ignores the HDS and (ii) the calculated dose number based on the VolSurf+ solubility and the HDS.[10].

A number of limitations of the procedures described in this report may explain the difficulties of proper BDDCS predictions: some are inherent to software, e.g., *in silico* tools rarely take self-association into account, as well as inherent limitations in solubility estimation; some may be related to the nature of the data collection process, e.g., the experimental solubility values are based on literature search and the data available did not always cover the entire range of pH between 1 and 7.5; finally, some are a consequence of the nature of the prediction, e.g., HDS not known. An easy way to understand the importance of HDS is to look at the performance of the PDN-MRKT descriptor (predicted dose number at HDS) on the same external validation set. When we assigned high solubility to drugs having PDN-MRKT equal or smaller than 1, there was a negligible increase in the model performance (78% versus 77%), with an increased accuracy for class 1 drugs (from 73% to 78%) and class 4 drugs (from 37% to 42%) (Figure 2 and Table S3 in the Supporting Information).

Extent of Metabolism Prediction. The three best models for EoM prediction were a naive Bayes using 4 variables, and two 5-variable SVM models using the polynomial and radial basis function kernel, respectively. For these 3 models, the classification accuracy was 81% for the training set and ~79% for the external validation set (Table S2 in the Supporting Information). The consensus model had a classification accuracy of 82% for the training set and 79% for the external set (Table S3 in the Supporting Information). The external validation performance was very good for class 2 (86%), reasonably good for classes 1 and 3 (around 75%), and poor for class 4 (63%). Three descriptors out of five selected for EoM modeling are related to the partition coefficient. It can be observed subsequently in Figure 7 that the experimental values for $LogD_{7.4}$ are reasonably well separated for class 4 compared to classes 1 and 2; however, the calculated values do not offer this separation. This once more indicates that lack of accurate predictions for class 4 drugs is not a consequence of our choice of descriptors or statistical tools, although poor $LogD_{7.4}$ calculations may have been a contributing factor. Once again, prediction performance was excellent for a class that was not included in the training set (class 2), confirming the validity of the strategy adopted.

Calculated BDDCS Class (Four-Class Prediction). The two consensus models were used to decide the final BDDCS class estimate. Various statistics for the internal and the external validation, comparisons with Chembench and earlier BDDCS models, and confusion matrices for the predictions are available in Figure 3, Figure 4, and Figure 5 and in Tables S5–S12 in the Supporting Information. Considering the first ranked BDDCS class prediction only, the model reported here is correct 69% for the training set and 55% for the external validation set (Figure 3A). The classification accuracy difference between the training and validation sets is due to the exclusion of class 4 drugs from the training set; when class 4 drugs were excluded from the test set, accuracy for the test set reaches 61%. When both the first and the second ranked BDDCS class probability were considered, the model was 94% correct in the training set and 92% in the external validation set (Figure 3A).

For the model based on separate FDAS and EoM predictions, single BDDCS class accuracy ranking in external validation corresponds to that from LDA modeling. Unsurprisingly, only class 4 was predicted with accuracy below 50%. In terms of average class accuracy the model was a marked improvement with respect to the RF and SVM 4-class models presented here, and to the earlier BDDCS modeling efforts (Figure 3B). In Figure 4 we show the accuracy in external prediction of each BDDCS class, for the earlier BDDCS models[17] and for the one presented in this work.

Calculated BDDCS Class (Single-Class Prediction). The probability of membership in a single BDDCS class derived from separate EoM and FDAS predictions can be used to categorize drugs belonging to a single targeted BDDCS class

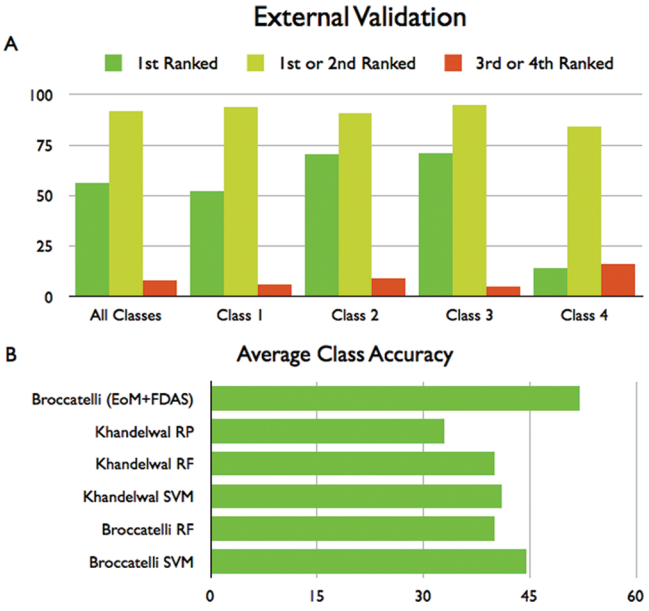

Figure 3. (A) Distribution of BDDCS predicted classes for the validation set (green for true class having highest predicted BDDCS class membership score, yellow for true class having predicted BDDCS class membership score within the 1st highest and the 2nd highest, red for incorrect classifications). (B) Comparison of the average class accuracy of different models for BDDCS class prediction.

(e.g., class 1 vs non-class 1 drugs). Binary classification for the targeted classes was also studied with the RF and SVM algorithms as implemented in Chembench. In Figure 5 we report ROC curves for single-class predictions.[30] The predictions based on the score resulting from the combination of FDAS and EoM estimation are referred to as "four-class model", while the best between the Chembench RF and SVM models for binary predictions are referred to as "two-class model". Results for class 4 are not shown, since none of the attempts reported here produced good predictions for this class.

BDDCS Forecast in Early Drug Discovery. To illustrate the potential application of BDDCS in early drug discovery, we report the results of BDDCS consensus model estimation on several categories of compounds, taken from a public data set.[23] This set included compounds in the drug development phase (I, II, III and launched; 3,464 compounds in total), as available in 2006; and compounds from the medicinal chemistry literature, as indexed in WOMBAT.[31,32] The WOMBAT compounds were separated into high-activity (W9), composed of 5,001 compounds for which the biological activity is below 1 nM, or above 9 units on the $-\log_{10}$ (activity) scale, in at least one of the documented literature assays; and low-activity (W6), which had 28,912 compounds for which the biological activity is above 1 μM, or below 6 units on the $-\log_{10}$ (activity) scale, on all of the documented literature assays. Excluded from the original data set were molecules with MW > 700 amu. Final BDDCS scores, as derived from the FDAS and EoM consensus models, are given in Table S19 in the Supporting Information. The BDDCS score by compound category is summarized in Table 1 and Figure 6. In our previous report,[10] we estimated the distribution by BDDCS class of all NMEs synthesized with the intent to make drugs. The data here are a better representation of molecules tested at some point for activity, since many very poorly soluble class 2 and 4 compounds do not even get to this stage.

Descriptor Analysis. A number of descriptors—some of which were not included in the final consensus models—uncovered differences among the BDDCS classes. For example, the predicted VolSurf+ plasma protein binding percentage (% PPB_V) seems to differentiate between classes 1, 2, and 3. By observing the distribution of the different classes according to the measured percentage of plasma protein binding (%PPB; see Figure 7), this differentiation is clearer. Experimental values for

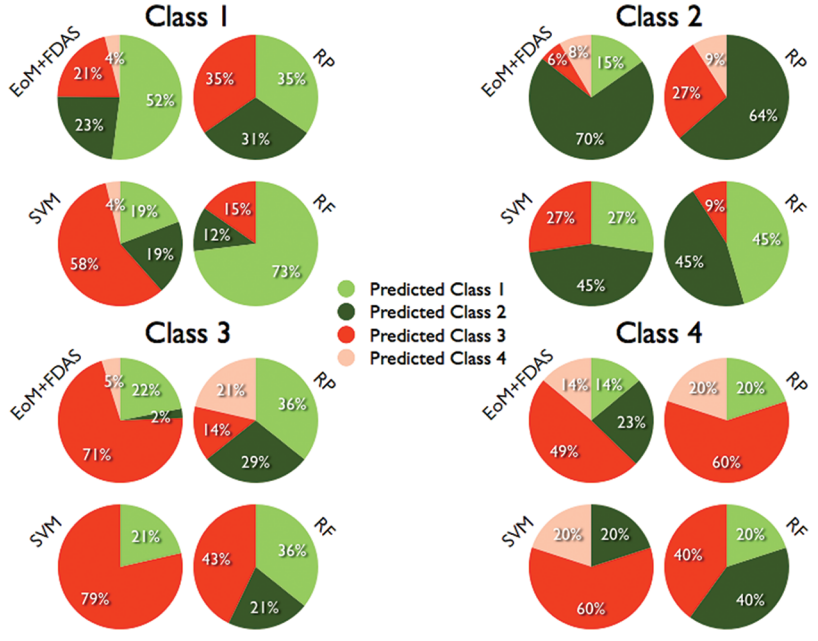

Figure 4. Pie charts showing the proportion of predicted BDDCS classes for each actual BDDCS class; predictions are shown for the consensus model presented in this work, based on separate estimations of EoM and FDAS, in comparison to the classification approaches (RP, SVM and RF) presented in Khandelwal et al.[17].

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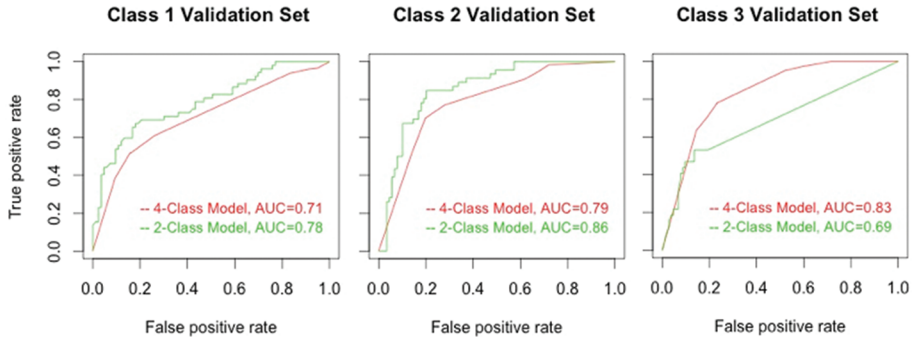

Figure 5. Single BDDCS class prediction for the validation set based on (i) predicted BDDCS class membership scores to each BDDCS class (4-class models derived from EoM and FDAS predictions) and (ii) 2-class models (either SVM or RF) targeting one specific class.

Table 1. BDDCS Class Prediction for Compounds in Different Drug Discovery Stages[a]

	count	class 1	class 2	class 3	class 4
oral drugs					
predicted[b]	679	0.31	0.34	0.29	0.06
actual[b]	679	0.41	0.32	0.21	0.06
drugs[c]	1557	0.43	0.21	0.31	0.05
phase 1[d]	713	0.39	0.33	0.23	0.05
phase 2[d]	922	0.41	0.33	0.21	0.05
phase 3[d]	272	0.36	0.30	0.29	0.05
W6[e]	28912	0.18	0.54	0.22	0.06
W9[e]	5001	0.31	0.53	0.12	0.05

[a]Data are presented as fraction of the count. [b]Oral drugs used to train and validate the model. [c]Drugs data set including both oral and nonoral drugs.[23] [d]Drug that accessed clinical phases.[23] [e]Medicinal chemistry compounds tested for at least one target and having micromolar (W6) or nanomolar (W9) bioactivity.[23,31,32]

623 BDDCS classified drugs were taken from WOMBAT-PK.[31,32] Class 2 drugs tend to be highly bound, whereas class 3 drugs are markedly more unbound. In particular, if 75% is considered as the cutoff for %PPB, approximately 85% of both classes 2 and 3 are correctly predicted. In agreement with the work of Kratochvil et al.,[33] LogP and %PPB show similar distributions with respect to BDDCS classes. Oversimplifying, %PPB could be seen as a partition between a water solution and a lipophilic environment (plasmatic proteins). While pharmacophore descriptors may be required to predict %PPB at the submicromolar level, the relatively simple descriptor framework of BDDCS can distinguish well between high and low %PPB.

As expected by the high degree of overlap between the BCS and BDDCS frameworks, the distribution of highly metabolized vs poorly metabolized drugs with respect to predicted VolSurf+ CACO2 (passive) permeability is also different (Figure 7). Since the VolSurf+ CACO2 permeability descriptor is derived from passive Caco-2 permeability, it was not intended to predict active transport. It appears that this "limitation" may actually help distinguish extensive metabolism from poor metabolism among drugs. The VolSurf+ CACO2 permeability model clearly distinguishes extensive from poor metabolism, but unlike octanol/water partition (either LogP or $LogD_{7.4}$), it has a similar distribution for class 1 and class 2 drugs (Tables S15 and S17 in the Supporting Information). For the VolSurf+ descriptor LgBB (predicted blood–brain barrier permeability), an intermediate distribution compared to LogP and the calculated CACO2 permeability was observed: LgBB appears to be slightly higher for class 1 vs class 2, and it is markedly higher for extensive metabolism vs poor metabolism (i.e., classes 1 and 2 vs 3 and 4, respectively). This differentiation is

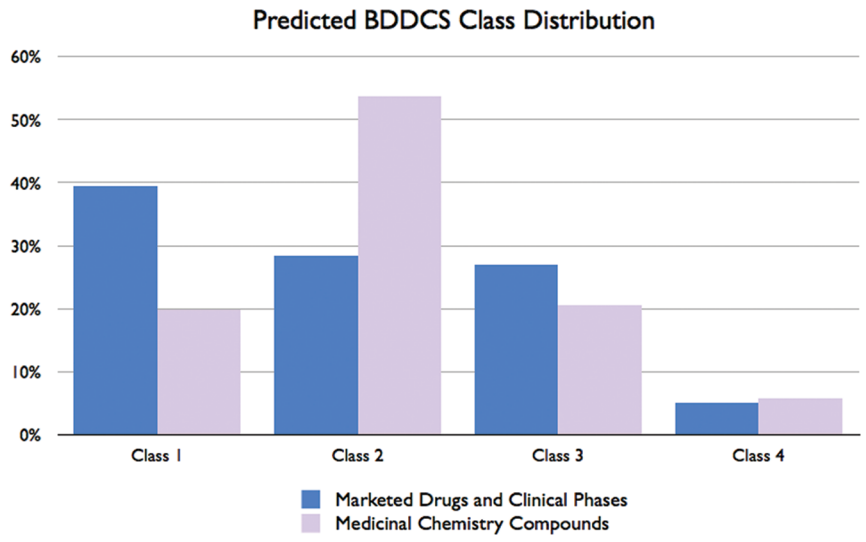

Figure 6. Predicted BDDCS class distribution for marketed and clinical drugs (oral, nonoral, clinical phases 1, 2 and 3) and medicinal chemistry compounds (extracted from WOMBAT).[23]

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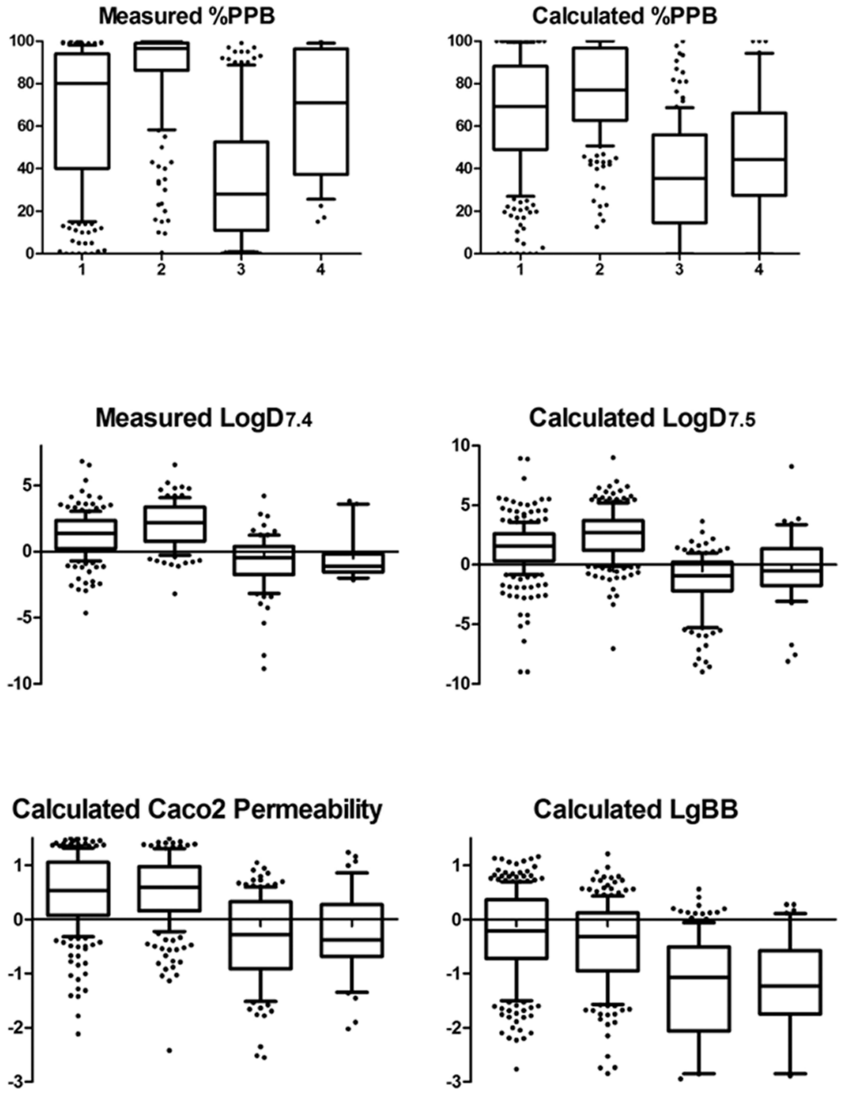

Figure 7. Box plots for measured and calculated plasma protein binding (PPB) percentages, measured $LogD_{7.4}$, calculated $LogD_{7.5}$, calculated Caco2 permeability and LgBB (predicted blood−brain barrier permeability). The calculated parameters are VolSurf+ descriptors. See Figure 1 for box plot description. Values for the 10th, 25th, 75th and 90th percentiles, together with number of molecules used, standard deviations, median and averages, are available in Tables S13−S18 in the Supporting Information.

supported by experimental data, as discussed in a recent report.[34]

When predicting FDAS, a certain number of descriptors were directly linked to (i) solubility calculation (LgS3, LgS4, LgS5, LgS6, LgS7.5, minVSLgS7.5) and (ii) solubility profiling (L3LgS), (iii) predicted dose numbers (PDN-MDN, PDN-AVG, PDN-75), (iv) descriptors for octanol/water partitioning (LgD5, LogP n-oct) and (v) protonation state (%FU9), respectively. All these descriptors were deemed relevant for FDAS (Table S2 in the Supporting Information).

DISCUSSION

In this report we describe a computational procedure to predict the BDDCS class of NMEs based on 3D-MIF descriptors. The model is based on our recently published compilation of BDDCS applied to over 900 drugs.[10] Mostly due to dose estimation requirements, the data set was reduced to 679 oral drugs, which were employed for training a consensus model (300 drugs), and for validating it (379 drugs). As outlined earlier, BDDCS is a four-class system based on two parameters: EoM and FDA solubility. Any attempt to model these two properties from molecular structure will have a number of difficulties and limitations. The first is that FDA solubility is a property of the drug formulation, and not a property of the API molecule itself. Second, FDAS is linked to the HDS, which is a complex property to model: HDS is dependent on several independent factors, such as the affinity to drug targets, other bioactivities on targets and off-targets, the volume of distribution at steady state, and toxicity, to name a few. As previously shown, it is important to consider the effects of pH since the FDA definition of solubility takes this into account.[2] Thus, for a more accurate FDAS prediction both pK_a and thermodynamic solubility predictions are required; this further assumes that the influence of HDS is minimal. Third, regarding the elimination route, the large overlap between BDDCS and BCS suggests that humans use different ways to eliminate permeable APIs as opposed to nonpermeable APIs. The descriptors used in this work (i.e., ligand-based, nonpharmacophoric descriptors that do not capture the influence of protein structures) are adequate for modeling ADME properties like

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solubility and permeability, and are rarely tailored to model ligand−protein interactions.[35] We are aware that in a number of cases the affinity of the ligand to the DMEs remains a determining factor, especially for drugs with borderline physicochemical properties; hence, by using descriptors that ignore the directionality, geometry, and specificity of the drug−DME binding, another intrinsic limitation is added.

The nature of the data set should also be considered: the 697 drugs are not equally distributed in the four-classes (41% class 1, 32% class 2, 21% class 3 and 6% class 4). Strictly based on our theoretical evaluation, BDDCS is unevenly distributed among NMEs as well (Table 1). Our preliminary LDA studies indicate that class 2 and class 3 drugs' properties are at different ends of the property distribution spectrum, whereas class 1 drugs are in between. Not surprising, LDA did not discriminate class 4 drugs from the rest of the data set. Due to the relatively small number of class 4 drugs and their limited data availability, we could not investigate this issue exhaustively, however we suggest that self-association in water could be one possible reason. This hypothesis is supported by Ross and Riley,[15] who demonstrated failure in predicting solubility for a number of class 4 drugs based on experimentally determined melting point and $LogD_{7.4}$ values. Predictions were inaccurate in the pH range where these drugs were zwitterions. Two facts from this study suggest that solubility prediction issues are a consequence of self-association in water: (i) the lowest experimental solubility was found in the pH range where the molecules have the highest number of ionized centers (while the opposite would be expected); (ii) in the pH range where solubility predictions were inaccurate, solubility could be increased by increasing the temperature. Accurate predictions and no temperature effect were observed when drugs were not zwitterions.

To avoid a classification scheme biased toward one class, we have developed separate models for FDAS and EoM and trained them with an equal number of randomly selected drugs from classes 1, 2, and 3. The FDAS model was trained using 100 class 1 and 100 class 2 drugs, whereas the EoM model was trained using 100 class 1 and 100 class 3 drugs. The validity of this strategy was confirmed by the reasonably good prediction accuracy in estimating EoM for class 2 drugs and FDAS for class 3 drugs, as each set was *not* included in the specified training sets.

The external prediction accuracy for EoM and FDAS was 79% and 77%, respectively. Not considering class 4 drugs, accuracy was above 80% for both properties. These predictions result from two consensus models based on 17 highly informative VolSurf+ descriptors. Three different classifiers "voted" for the final prediction of both properties; their degree of agreement was used in the final classification, as part of the score. The classifiers used were naive Bayes, kNN and SVM; none of them significantly outperforms the others in this data set. Considering HDS in the calculations did not significantly change the overall model performance, but led to more balanced predictions for classes 1 and 3 (from 73% and 91% to 78% and 90%, respectively).

The final prediction was given as a probability of membership in each BDDCS class. In over 92% of the cases, the actual class had either the highest or the second highest predicted BDDCS class membership score. This suggests that, by experimentally testing either one of these properties, it could be possible to properly assign the BDDCS class for NMEs with a confidence of 90% or above. The models based on EoM and FDAS predictions were not biased toward any one BDDCS class, as opposed to using the RF or SVM algorithms in a single step 4-label classification model. As expected based on the LDA model, the predictions were accurate for class 2 and class 3 drugs, less accurate for the most populated BDDCS class 1 and extremely poor for the BDDCS class 4 drugs. When using the calculated probability of membership for predicting a BDDCS class versus the others, the accuracy was 83% for class 3, 77% for class 2 and 69% for class 1. Using RF or SVM models in single-class mode, the classification accuracy increased for the more populated classes 1 and 2 (76% for the class 1 RF model and 82% for the class 2 RF model) and decreased for class 3. If it is of interest to prioritize the compounds more likely to belong to a particular class, the use of RF binary models for classes 1 and 2 is recommended. For class 3, it is better to use the EoM and FDAS based probability models. For BDDCS class 1, it might be more relevant to have good precision (i.e., higher chances that the prediction is correct) instead of accuracy; in other words, it could be of interest to predict the highest fraction of the BDDCS class 1 molecules with high confidence. This may be relevant since NMEs attributed to class 1 are not likely to be affected by transporters *in vivo* and therefore should not be tested *in vitro*. By increasing the threshold values used for BDDCS class 1 predictions in the RF binary model, it is possible to predict 52% of the class 1 drugs in the validation set with a confidence of 87%. Concerning the EoM prediction, the EoM class is correctly predicted in external validation with a confidence of 87% when the first and the second ranked BDDCS classes in predictions belong to the same metabolic class (e.g., BDDCS classes 1 and 2).

In this work we presented strategies that improve the total accuracy, the average class accuracy, and the single class predictions in respect to earlier BDDCS models. With respect to total accuracy, we showed that by using a SVM model it is possible to have 64% accuracy in external class predictions, but we also showed that the same model is only 44% accurate when the average class accuracy is considered. This imbalance is the result of the uneven distribution of drugs in the BDDCS classes.

We found a significant difference between the BDDCS class distribution for drugs, that predicted for phases 1, 2, and 3 (e.g., ∼39% are class 1; ∼32% class 2; and ∼25% class 3), and the BDDCS prediction for medicinal chemistry compounds from WOMBAT (∼20% are class 1; ∼54% class 2; and ∼20% class 3). We estimate that more than 60% of the NMEs evaluated in early drug discovery are poorly soluble compounds (most likely BDDCS class 2) regardless of bioactivity, since a similar percentage of poorly soluble compounds was found in the W6 (low bioactivity) and the W9 (high bioactivity) data sets (Table 1 and Figure 7). We emphasize that these published compounds embody success stories from early drug discovery, and thus they may represent only a small fraction of the actual chemical libraries of pharmaceutical companies.

Since early phases of drug discovery are mainly oriented on achieving good bioactivity, the prevalence of class 2 NMEs for the W6 and W9 sets suggests a tendency to be active despite the biological target for this class. Therefore class 2 NMEs are more likely to represent the most promiscuous BDDCS class, possibly due to nonspecific lipophilic interaction. In humans, this translates into a higher risk of toxicity, especially when metabolism is altered. Recall that BDDCS class 2 hERG inhibitors astemizole, cisapride and terfenadine were withdrawn from the market as a consequence of overdose or metabolism alteration.[36] Given that our models do not overestimate

BDDCS class 2 assignment for oral drugs, this data substantially supports our previous forecast on the difference in BDDCS class distribution for NMEs being developed by the industry vs marketed oral drugs.[10] Because we anticipate the distribution of NMEs in BDDCS categorization to be very different from the one observed in approved oral drugs given our findings with the WOMBAT data set, we discourage the use of total accuracy for judging BDDCS class predictions; rather, we suggest the use of the average class accuracy, a more suitable metric.

CONCLUSIONS

In this work we present several strategies and models for predicting BDDCS classification. The best model for 4-class predictions is based on predicting EoM and FDAS separately, which are then combined to produce scores for the probability of membership in each BDDCS class. The performances and the robustness of these models appear to have increased compared to previous *in silico* BDDCS models. Predictions can also be used to estimate FDAS and EoM. The EoM model is based on *in vivo* data and is also suitable to forecast DDIs.[13,37]

Predictions for EoM and FDAS are over 80% accurate when the least represented BDDCS class 4 is not considered. For BDDCS class 4 drugs these models generally fail. In an early drug discovery setting, we encourage scientists to combine these computational models with *in vitro* passive permeability and solubility assays, in order to increase the accuracy of BDDCS classification. In this manner, BDDCS class prediction would avoid the limitations discussed here, and make the best use of the models presented in this work.

BDDCS class prediction can help to prioritize *in vitro* experiments for NMEs in order to (i) test only NMEs and transporters that are relevant *in vivo* and (ii) anticipate drug−drug interactions before entering clinical trials. The latter point is of particular importance, since adverse drug reactions are often hard to detect, even during clinical trials.[9] The use of BDDCS in predicting the role of transporters in the disposition of marketed drugs, and the potential drug−drug interactions derived by their modulation, has been widely investigated and reviewed in detail elsewhere.[6] Industry is now recognizing the potential of using BDDCS during early drug discovery phases. Varma and collaborators suggested that BDDCS class 3 NMEs are likely to target intestinal transporters in order to optimize their intestinal absorption.[38] Recently we showed that BDDCS class 1 NMEs are ideal CNS candidate drugs and we presented several BDDCS based strategies to be employed in early phases of drug discovery.[34]

Based on BDDCS, the intestinal absorption of extensively metabolized drugs is likely to be optimal and not affected by uptake transporters. By using predicted EoM it could be possible to identify molecules for which passive permeability testing is not necessary. For these molecules the modulation of metabolic enzyme activity could result in severe adverse drug reactions. In contrast, NMEs predicted to be poorly metabolized should target uptake transporters, in order to optimize their intestinal absorption. This awareness could allow scientists to forecast DDIs with therapeutics that induce or inhibit uptake transporters. When the first and the second ranked BDDCS classes belong to the same metabolic class, EoM predictions are correct 87% of the time.

BDDCS class 1 NMEs are optimal CNS candidates.[34] By using a RF binary model for BDDCS class1 NME predictions it is possible to identify BDDCS class 1 drugs with an accuracy of 76%. Alternatively, if it is of interest to minimize the number of false positive predictions, the model can be used with a different threshold to predict 52% of the BDDCS class 1 NMEs with a confidence of 87%. BDDCS class 2 NMEs can be confidently predicted by the models presented. For these molecules transporters are not expected to influence intestinal absorption, but might lower the brain disposition. Nevertheless, if these compounds are extensively metabolized by enzymes in the gut, efflux transporters might be a major determinant of their metabolism. Therefore, if class 2 NMEs are CNS candidate drugs or are metabolized in the gut, they should be tested *in vitro* for efflux transporters. By recognizing the enzyme−transporter interplay before clinical phases, it could be possible to anticipate *in vivo* DDIs.[7,8] The properties of BDDCS class 2 NMEs warrant closer investigation, as they appear to represent over 50% of the medicinal chemistry compounds from early drug discovery regardless of bioactivity, based on a representative literature sample.

The inability to predict the under-represented BDDCS class 4 is a limitation of this model, which based on extensive analyses is linked to the quality of the data set. Schuster and colleagues showed that the fraction of candidate drugs failing during clinical phases due to poor ADME properties is relatively small, as opposed to the number of candidate drugs failing clinical trials due to toxicity issues.[9] Hence, while industry has already developed effective tools to identify NMEs with an unfavorable ADME profile (e.g., class 4), further improvements must be done to predict NMEs' disposition and toxicity with respect to the interaction with biological modulators (transporters, metabolizing enzymes, plasma proteins, antitargets). These models might provide a rational approach to face this challenge.

ASSOCIATED CONTENT

Supporting Information

Table S1 lists data set drugs and calculated descriptors. Table S2 lists the descriptors considered and/or used for each model. Table S3 lists performances for single properties models and the options used. Table S4 lists predicted FDAS, EoM and BDDCS class probability. Tables S5, S6, S7, S8, S9, S10 and S11 list the performances in training and validation set for predicting BDDCS classes. Table S12 lists the performances of different models for BDDCS prediction. Tables S13, S14, S15, S16, S17 and S18, list 10th, 25th, 75th and 90th percentiles, number of molecules used, standard deviations, medians and averages for several calculated and measured properties. Table S19 lists the BDDCS class predictions for the large medicinal chemistry data set. Table S20 shows results of the Chembench models and the LDA. Figures SA, SB and SC show false positive rate vs classification accuracy in single-class screening for BDDCS classes 1, 2, and 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

BDDCS, Biopharmaceutics Drug Disposition Classification System; BCS, Biopharmaceutics Classification System; DDI, drug−drug interaction; EoM, extent of metabolism; FDAS, FDA solubility; NME, new molecular entity; HDS, highest dose strength; DME, drug metabolizing enzyme; API, active pharmaceutical ingredient; ADMET, absorption−distribution−metabolism−excretion−toxicity; MIF, molecular interaction fields; DN, dose number; PDN, predicted dose number; PDN-MRKT, predicted dose number at highest dose strength; kNN, *k* nearest neighbor; CNS, central nervous system; % PPB_V, VolSurf+ plasma protein binding percentage; %PPB, plasma protein binding percentage

■ REFERENCES

(1) Amidon, G. L.; Lennernas, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res.* **1995**, *12*, 413−420.

(2) Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. *FDA Guidance for Industry*; Food and Drug Administration: Rockville, MD, 2000.

(3) Wu, C. Y.; Benet, L. Z. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm. Res.* **2005**, *22*, 11−23.

(4) Benet, L. Z.; Amidon, G. L.; Barends, D. M.; Lennernas, H.; Polli, J. E.; Shah, V. P.; Stavchansky, S. A.; Yu, L. X. The use of BDDCS in classifying the permeability of marketed drugs. *Pharm. Res.* **2008**, *25*, 483−488.

(5) Committee for Medicinal Products for Human Use (CHMP). *Guideline on the investigation of bioequivalence*; European Medicines Agency: London, U.K., 2010.

(6) Shugarts, S.; Benet, L. Z. The role of transporters in the pharmacokinetics of orally administered drugs. *Pharm. Res.* **2009**, *26*, 2039−2054.

(7) Benet, L. Z. The drug transporter-metabolism alliance: uncovering and defining the interplay. *Mol. Pharmaceutics* **2009**, *6*, 1631−1643.

(8) Benet, L. Z.; Cummins, C. L.; Wu, C. Y. Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int. J. Pharm.* **2004**, *277*, 3−9.

(9) Schuster, D. L., C.; Langer, T. Why Drugs Fail—A study on side effects in new chemical entities. In *Antitargets Prediction and Prevention of Drug Side Effects*; Vaz, R. J., Klabunde, T., Eds.; Wiley-VCH: 2008; pp 3−22.

(10) Benet, L. Z.; Broccatelli, F.; Oprea, T. I. BDDCS applied to over 900 drugs. *AAPS J.* **2011**, *13*, 519−547.

(11) Takagi, T.; Ramachandran, C.; Bermejo, M.; Yamashita, S.; Yu, L. X.; Amidon, G. L. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol. Pharmaceutics* **2006**, *3*, 631−643.

(12) Chen, M. L.; Amidon, G. L.; Benet, L. Z.; Lennernas, H.; Yu, L. X. The BCS, BDDCS, and regulatory guidances. *Pharm. Res.* **2011**, *28*, 1774−1778.

(13) VolSurf+, version 1.0.4; available from Molecular Discovery Ltd., London, U.K. (http://www.moldiscovery.com).

(14) Cruciani, G.; Crivori, P.; Carrupt, P.-A.; Testa, B. Molecular fields in quantitative structure-permeation relationships: the VolSurf approach. *J. Mol. Struct.: THEOCHEM* **2000**, *503*, 7−30.

(15) Ross, D. L.; Riley, C. M. Aqueous solubilities of some variously substituted quinolone antimicrobials. *Int. J. Pharm.* **1990**, *63*, 237−250.

(16) Goodford, P. J. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. *J. Med. Chem.* **1985**, *28*, 849−857.

(17) Khandelwal, A.; Bahadduri, P. M.; Chang, C.; Polli, J. E.; Swaan, P. W.; Ekins, S. Computational models to assign biopharmaceutics drug disposition classification from molecular structure. *Pharm. Res.* **2007**, *24*, 2249−2262.

(18) 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.

(19) Carosati, E.; Mannhold, R.; Wahl, P.; Hansen, J. B.; Fremming, T.; Zamora, I.; Cianchetta, G.; Baroni, M. Virtual screening for novel openers of pancreatic K(ATP) channels. *J. Med. Chem.* **2007**, *50*, 2117−2126.

(20) Ottaviani, G.; Martel, S.; Carrupt, P. A. *In silico* and *in vitro* filters for the fast estimation of skin permeation and distribution of new chemical entities. *J. Med. Chem.* **2007**, *50*, 742−748.

(21) Cross, S.; Cruciani, G. Molecular fields in drug discovery: getting old or reaching maturity? *Drug Discovery Today* **2010**, *15*, 23−32.

(22) Broccatelli, F.; Carosati, E.; Neri, A.; Frosini, M.; Goracci, L.; Oprea, T. I.; Cruciani, G. A novel approach for predicting P-glycoprotein (ABCB1) inhibition using molecular interaction fields. *J. Med. Chem.* **2011**, *54*, 1740−1751.

(23) Oprea, T. I.; Allu, T. K.; Fara, D. C.; Rad, R. F.; Ostopovici, L.; Bologa, C. G. Lead-like, drug-like or "Pub-like": how different are they? *J. Comput.-Aided Mol. Des.* **2007**, *21*, 113−119.

(24) Orange Canvas, version 2.0b (http://orange.biolab.si).

(25) Venables, W. N.; Ripley, B. D. *Modern Applied Statistics with S*, 4th ed.; Springer: New York, 2002.

(26) Tropsha, A. Best practices for QSAR model development, validation, and exploitation. *Mol. Inf.* **2010**, *29*, 476−478.

(27) Chembench, http://chembench.mml.unc.edu (accessed on Novermber 2010).

(28) Cortes, C.; Vapnik, V. Support-Vector Networks. *Mach. Learn.* **1995**, *297*, 273−297.

(29) Breiman and Cutler's Random Forests for classification and regression, version 4.5−18 (http://stat-www.berkeley.edu/users/breiman/RandomForests/cc_home.htm).

(30) Fawcett, T. ROC graphs: Notes and practical considerations for data mining researchers; Tech. report HPL-2003-4; HP Laboratories: Palo Alto, CA, 2003.

(31) Olah, M.; Rad, R.; Ostopovici, L.; Borá, A.; Hadaruga, N.; Hadaruga, D.; Moldovan, R.; Fulias, A.; Mracec, M.; Oprea, T. I. WOMBAT and WOMBAT-PK: Bioactivity databases for lead and drug discovery. In *Chemical Biology: From Small Molecules to Systems Biology and Drug Design*; Schreiber, S. L. K., Kapoor, T. M.; Wess, G., Eds.; Wiley-VCH: 2007; pp 780−786.

(32) WOMBAT-PK 2010, available from Sunset Molecular Discovery LLC, New Mexico, USA (http://www.sunsetmolecular.com).

(33) Kratochwil, N. A.; Huber, W.; Muller, F.; Kansy, M.; Gerber, P. R. Predicting plasma protein binding of drugs: a new approach. *Biochem. Pharmacol.* **2002**, *64*, 1355−1374.

(34) Broccatelli, F.; Larregieu, C. A.; Cruciani, G.; Oprea, T. I.; Benet, L. Z. Improving the prediction of the brain disposition for orally administered drugs using BDDCS. *Adv. Drug Delivery Rev.* **2012**, DOI: 10.1016/j.addr.2011.12.008.

(35) Zamora, I.; Oprea, T.; Cruciani, G.; Pastor, M.; Ungell, A. L. Surface descriptors for protein-ligand affinity prediction. *J. Med. Chem.* **2003**, *46*, 25−33.

(36) Aronov, A. Predictive *in silico* modeling for hERG channel blockers. *Drug Discovery Today* **2005**, *10*, 149−155.

(37) Lam, J. L.; Benet, L. Z. Hepatic microsome studies are insufficient to characterize in vivo hepatic metabolic clearance and metabolic drug-drug interactions: studies of digoxin metabolism in primary rat hepatocytes versus microsomes. *Drug Metab. Dispos.* **2004**, *32*, 1311−1316.

(38) Varma, M. V.; Ambler, C. M.; Ullah, M.; Rotter, C. J.; Sun, H.; Litchfield, J.; Fenner, K. S.; El-Kattan, A. F. Targeting intestinal transporters for optimizing oral drug absorption. *Curr. Drug Metab.* **2010**, *11*, 730−742.