

Use of Robust Classification Techniques for the Prediction of Human Cytochrome P450 2D6 Inhibition

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A new *in silico* model is developed to predict cytochrome P450 2D6 inhibition from 2D chemical structure. Using a diverse training set of 100 compounds with published inhibition constants, an ensemble approach to recursive partitioning is applied to create a large number of classification trees, each of which yields a yes/no prediction about inhibition for a given compound. These binary classifications are combined to provide an overall prediction, which answers the yes/no question about inhibition and provides a measure of confidence about that prediction. Compared to single-tree models, the ensemble approach is less sensitive to noise in the experimental data as well as to changes in the training set. Internal validation tests indicated an overall classification accuracy of 75%, whereas predictions applied to an external set of 51 compounds yielded 80% accuracy, with all inhibitors correctly identified. The speed and 2D nature of this model make it appropriate for high-throughput processing of large chemical libraries, and the confidence level provides a continuous scale on which to prioritize compounds.

INTRODUCTION

Out of approximately 250 compounds entering pre-clinical development, only five are tested in the clinic, and only one is ultimately approved by the FDA. ADME/Tox factors are cited in 70% of these failures,¹ so the early identification of problematic candidates has understandably become one of the highest priorities in the pharmaceutical industry. Early identification is commonly facilitated through the use of *in vitro* ADME/Tox screens or with the aid of *in silico* models. As more data become available, computer-based predictions have become more reliable, so *in silico* ADME/Tox models are an increasingly important element of the drug discovery paradigm.

Metabolism determines the fate of a compound entering the body, ultimately controlling whether that compound exerts a toxic effect. Ideally, drugs and other xenobiotics are broken down to harmless, soluble metabolites, which are easily excreted through the urine or bile. Occasionally, though, an otherwise innocuous substance is converted to a harmful metabolite in a process known as *metabolic toxification*.² Other compounds give rise to *drug–drug interactions*³ by inducing or inhibiting the activities of metabolic enzymes, impacting the normal detoxification or elimination of co-administered drugs. Because of their role in phase I metabolism, the cytochrome P-450 enzymes are implicated in many hundreds of cases of metabolic toxification² and clinically significant drug–drug interactions.³

The cytochrome P-450 family is composed of over 30 distinct isozymes with the majority of drugs being metabolized by a combination of the CYP3A4, CYP2D6, and CYP1A2 isoforms and members of the CYP2C subfamily.³ CYP2D6 has been studied extensively, partially because it

exhibits polymorphisms that affect significant fractions of the population. Approximately 7–10% of Caucasians⁴ and 1% of Asians⁵ lack CYP2D6 activity and are thus classified as *poor metabolizers* of CYP2D6 substrates. Interest in this isoform also stems from the fact that it catalyzes the oxidation of many broadly prescribed pharmaceuticals, including antiarrhythmics, antidepressants, antipsychotics, beta-blockers, and analgesics.⁶ This promiscuity with respect to substrates naturally makes CYP2D6 prone to inhibition by numerous drugs from these and other therapeutically important classes, setting the stage for clinically significant drug–drug interactions.³ Quinidine,⁷ clemastine,⁸ fluoxetine,⁹ terbinafine,¹⁰ and ritonavir¹¹ are just a few inhibitors of CYP2D6 which have the potential to block the metabolism of other drugs prescribed simultaneously.

Developing filters to identify probable inhibitors of CYP2D6 clearly provides a means of avoiding the pursuit of therapeutic candidates that ultimately may be responsible for these sorts of undesirable side-effects. *In silico* techniques of assessing inhibition potential are particularly attractive as they may be applied to entire chemical libraries at the outset of the drug discovery process, usually at a very small cost. We describe here a method for the prediction of CYP2D6 inhibition based only on knowledge of 2D chemical structure. This *in silico* model utilizes an *ensemble* approach to recursive partitioning,¹² providing both a yes/no prediction about inhibition and a confidence level that may be used to identify which predictions are most likely to be correct. A diverse set of compounds with published inhibition constants is employed in the development of this model and careful validation is carried out on these and other compounds to establish the reliability of the predictions.

Data Collection. An extensive literature search was carried out to locate compounds with reported K_i values for inhibition of human CYP2D6. Published *quantitative* data for this isoform are not particularly abundant, so a fairly relaxed set

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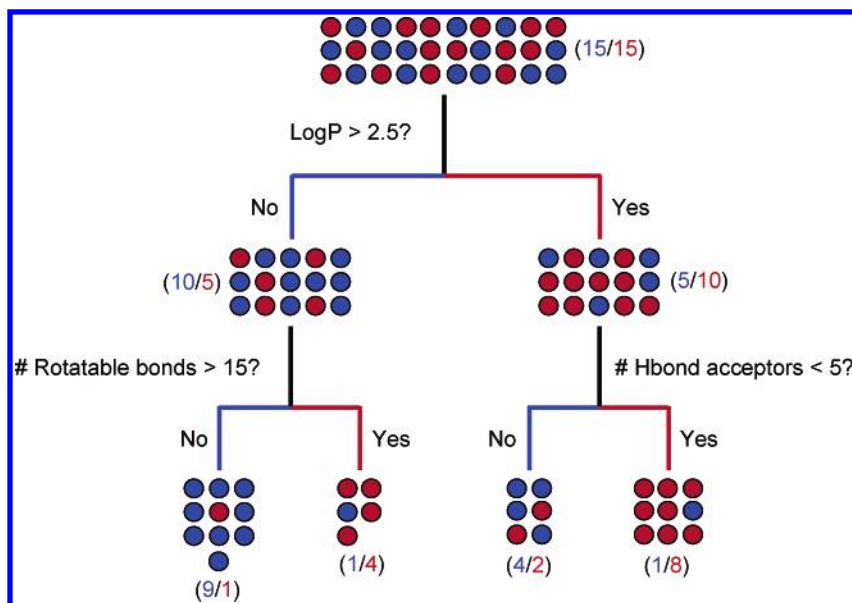


Figure 1. A decision tree that splits a training set of 30 compounds into four groups (leaves) that are purer in composition than the overall training set.

of criteria were employed throughout the search. For example, many different substrates were permitted, as were various types of inhibition, including competitive, partial competitive, mixed, and noncompetitive.¹³ This process yielded a relatively small but chemically diverse training set of 100 drugs, drug-like compounds, and naturally occurring chemicals, spanning molecular weights from 127 to 721, with K_i values between 0.0048 μM and 1000 μM .^{8,14–40}

Considering the range of substrates and inhibition types, as well as the potential for interlaboratory variations in experimental protocol, it was decided that a categorical inhibition model would be most appropriate. Accordingly, an active/inactive cutoff was set at 10 μM , yielding 59 inhibitors and 41 noninhibitors. Although any particular threshold is necessarily somewhat arbitrary, 10 μM is on the order of the expected hepatic blood concentration of typical drugs when administered at therapeutic doses.⁸

After collection of this training set and development of the *in silico* model, a second dataset of 51 compounds was assembled from published sources^{24,25,41–64} using the same criteria described above. This *external validation* set contained 10 inhibitors and 41 noninhibitors from numerous chemical families spanning molecular weights from 114 to 875. As these compounds were gathered independently from the training set, they offer a reasonably unbiased assessment of how the model can be expected to perform in the general sense.

Computational Model. After deciding on the nature of the endpoint prediction (continuous vs categorical), the next issue is whether to subdivide the training set into fairly homologous groups of compounds, developing a separate model on each subset. Though it is common practice to do such a subdivision, models derived therefrom may suffer from poor predictivity, especially when applied to compounds that do not fit clearly into one particular model subset. Subdividing the training set also increases the chances of over-fitting, simply because models are developed on smaller, less diverse sets of compounds in which chance correlations are more likely to occur.

To avoid these pitfalls, we consider all available training set data at once and employ an *ensemble* technique,¹² wherein predictions are made using an average of numerous models developed from random subsets of the training set. Whereas individual models may contain biases and defects stemming from noise and over-fitting, the average model can be expected to exhibit an improved signal-to-noise ratio and therefore more accurate predictions. An ensemble approach may be applied to any of the various model-building techniques,¹² but we choose recursive partitioning^{65,66} in the present case because it does not assume any particular functional relationship between the endpoint and the independent variables, and as such it is one of the most powerful classification methods available.

Recursive partitioning (RP) involves the creation of a decision tree which poses a series of yes/no questions about the values of various independent variables in a manner that splits a training set into progressively smaller groups that have a higher “purity” with respect to some property of interest. For CYP2D6 inhibition, the goal is to break up the training set into groups of compounds that are either predominantly inhibitors or predominantly noninhibitors. Figure 1 illustrates a hypothetical decision tree that might be built to purify a training set of 30 compounds falling into one of two categories (red, blue).

At each splitting point or node, the RP algorithm searches a pool of independent variables (i.e., descriptors) and identifies a single variable and corresponding splitting value that best purifies the group of compounds entering the node. The splitting process continues until either no further improvement can be achieved, or the numbers of compounds in each purified group are too small to justify further splitting. At that point, the training set has been filtered into a final collection of terminal nodes or *leaves*, each of which is identified with the class that predominates in the population.

Once a decision tree is constructed from a training set, it can be applied to any compound for which the necessary descriptors are known. The query compound is ultimately filtered into one of the leaves, and a prediction of its class

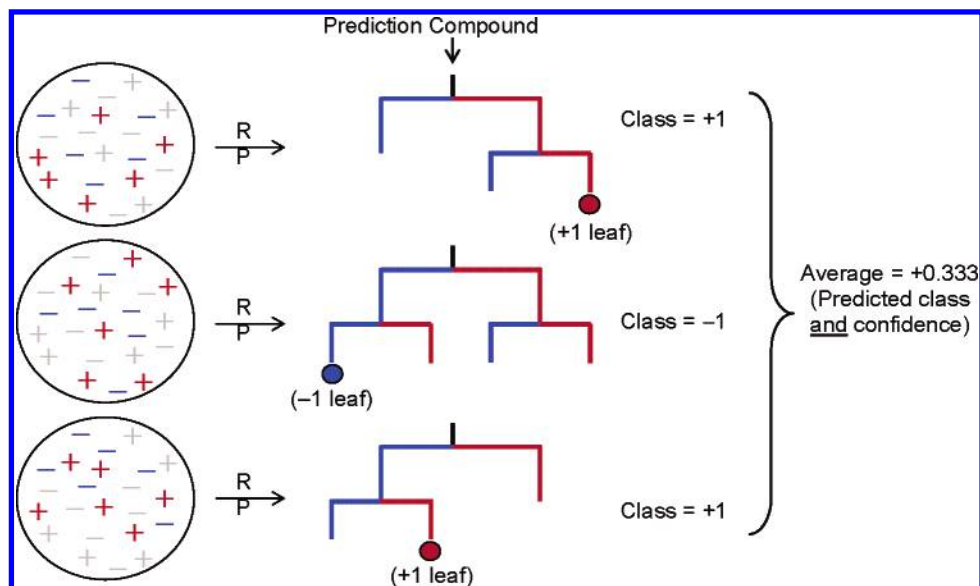


Figure 2. Ensemble approach to recursive partitioning. The red “+” and blue “-” characters indicate the members of the training set that are randomly selected and used to build each tree.

(red/blue, inhibitor/noninhibitor, etc.) is made according to the identity assigned to the leaf when the tree was built.

Although quite powerful, RP can be readily abused. Often, it is relatively easy to identify descriptors and splitting criteria that purify a training set, but if conditions are not carefully controlled, the resulting decision tree may yield poor predictions when applied to new compounds. Some contributing factors include the use of descriptor pools that are too large, allowing leaves to become too small, and employing training sets that are overly biased toward one class. RP is also sensitive to the composition of the training set, so that removal of even one observation has the potential to drastically change the resulting decision tree.

The techniques employed here address all of these issues and provide predictions that not only indicate the class of a compound, but also a level of confidence about that prediction. Figure 2 illustrates the basic principles behind the ensemble RP approach. At each iteration, the model-building algorithm selects from the training set a random subset containing equal numbers of positives (+1) and negatives (-1), where positives are inhibitors. A tree is built using only those compounds and it is stored for later use. Once a sufficiently large number of trees has been built, they can be applied sequentially to any query compound, yielding a series of ± 1 predictions, depending on whether the query is filtered into a +1 leaf or a -1 leaf. If these ± 1 values are summed and divided by the number of trees, an average class is afforded. The algebraic sign indicates the category into which the compound should be placed (positive/negative), and the absolute value indicates the level of confidence.

As stated previously, this approach automatically attenuates noise and defects of individual models. There is no doubt that a given tree is partially invalidated by errors in the experimental data, failure of certain descriptors to accurately model the property being predicted, and incomplete coverage of chemical space by the training set. However, these factors tend to bias trees in random ways, so when an ensemble approach is employed, the biases tend to cancel each other out, leaving an average model that is largely free of errors

arising from statistical artifacts. This sort of model can be expected to have general applicability and high predictivity.

Our ensemble approach may be compared to that of *bagging*,⁶⁷ but with the distinction that each sampled training set contains equal numbers of positives and negatives, and that no observation appears more than once in the sample. Bagging assigns all observations an equal probability of being selected, so the sampled populations will generally have the same distribution of positives and negatives as the overall training set. Bagging also utilizes sampling *with replacement*, so certain observations may appear more than once in a given subset.

Computational Details. Several hundred 2D structural descriptors were initially computed for the training set. These included Cerius² topological, electrotopological, and physicochemical parameters, various fragment keys based on AlogP atom types,⁶⁸ and 1D similarity scores⁶⁹ computed against each member of the training set. As discussed previously, RP models may exhibit low predictivity when derived from an excessively large descriptor pool. Accordingly, this rather enormous collection of descriptors was pared down to a manageable set of 25 using an in-house Monte Carlo simulated annealing algorithm designed to produce a 25-variable linear least-squares fit of a ± 1 inhibition variable.

In this approach, an initial set of 25 descriptors was chosen using simple forward stepwise selection, which involved nothing more than identification of the best 1-variable model, followed by sequential addition of 24 descriptors, with no deletions. In other words, the n th descriptor added was the one that yielded best n -variable fit, given that the previous $n - 1$ descriptors remained in the model. This provided a starting point for the Monte Carlo simulated annealing algorithm. Here, individual descriptors in an evolving 25-variable model were selected at random and flagged for possible replacement by a descriptor in the unused portion of the large pool. The candidate chosen from the pool was the descriptor that yielded the best 25-variable fit when combined with the other 24 descriptors still in the model.

So while a random scheme was used to select the descriptor for ejection, a greedy scheme was used to identify its possible replacement. If the new model produced a lower standard deviation of regression, the replacement was made; if not, the Monte Carlo test was applied. The temperature was reduced over the course of 1000 steps in a log-linear fashion. Upper and lower limits were set at $0.5\sigma_y$ and $0.05\sigma_y$, respectively, where σ_y was the observed standard deviation in the ± 1 inhibition variable.

The filtered pool of 25 descriptors was submitted to both the ensemble RP approach and the standard, single-tree approach. All decision trees were generated with the aid of the S-PLUS statistical package,⁷⁰ using default settings unless otherwise noted. No pruning was performed, but, as discussed below, minimum node size was controlled according to the results of leave-one-out experiments.

In the ensemble tree-generation procedure, subsets containing 35 inhibitors and 35 noninhibitors were selected randomly from the overall training set. A total of 101 subsets were selected this way, yielding 101 decision trees. Although some characteristics were found to be common to more than one tree, no single pattern was shared among all trees. An odd number of trees was employed so that the average class would never be exactly 0.0. We determined that 101 was also sufficient to yield satisfactory convergence in the average class values.

To validate the methodology and models, both internal and external validation experiments were performed. Internal validation involved a leave-one-out (LOO) procedure, wherein each of the 100 training set compounds was held out and predicted using decision trees built from the remaining 99 compounds. This technique was applied to both the ensemble and standard, single-tree models. Note that each LOO compound has no influence on the particular descriptors and splitting criteria selected during tree generation, so these tests are indicative whether the ensemble and standard RP approaches are capable of producing predictive models. This amounts to a validation of the methodology itself, and not a validation of any particular model. LOO tests may also be used to arrive at appropriate settings for parameters that control the size and complexity of each tree. For example, the minimum allowed node size was optimized by monitoring its effect on the accuracy of the LOO predictions.

Final model validation was accomplished by performing predictions on the external set of 51 compounds, using ensemble and standard RP models derived from the full 100-member training set. As noted previously, these 51 compounds were not present during any phase of model development, and therefore represent a pure external prediction set.

RESULTS AND DISCUSSION

The Monte Carlo filtering procedure yielded the pool of 25 descriptors shown in Table 1. This set was used to generate all LOO results as well as the final standard and ensemble RP models. A fairly wide range of descriptors is observed, although 13 of the 25 are 1D similarities to specific members of the training set. The similarities provide a convenient means of determining whether a given compound is likely to possess various structural characteristics that are critical for inhibition.

Table 1. Filtered Pool of 25 Descriptors

descriptor	definition
Atype_C_6	AlogP atom type counts ⁶⁸
Atype_C_11	
Atype_C_44	
Atype_O_56	
Atype_O_57	
I_sF	indicator variables for presence of fluorine and iodine ⁶⁸
I_sI	
S_sssCH	sum of E-State values for methine carbons ⁶⁸
SC-1	number of 1st order subgraphs ⁶⁸
Chiralcenter	number of chiral centers ⁶⁸
CHI-V-3_C	valence-corrected 3rd order cluster connectivity index ⁶⁸
MolRef	molar refractivity ⁶⁸
SIM1D(Nelfinavir)	1D similarity to specific training set compounds ⁶⁹
SIM1D(Venlafaxine)	
SIM1D(Nefazodone)	
SIM1D(9-Epiquinidine)	
SIM1D(Haloperidol)	
SIM1D(Chloroquine)	
SIM1D(Tetrahydroalstonine)	
SIM1D((R,R)-Reboxetine)	
SIM1D(Aprindine)	
SIM1D(LY237733)	
SIM1D(Diphenhydramine)	
SIM1D(Hyperforin)	
SIM1D(LY333531)	

Table 2. Summary of Leave-one-out (LOO) Predictions

RP method	correct classifications		
	inhibitors	noninhibitors	all
ensemble	48/59 (81%)	27/41 (66%)	75/100 (75%)
standard	38/59 (64%)	24/41 (59%)	62/100 (62%)

Systematic variation of the minimum allowed node size in the LOO experiments indicated that near optimal predictivity occurred when nodes with fewer than 10 compounds were not subjected to further splitting (i.e., the S-PLUS MINSIZE parameter was set to 10). This condition was observed for both ensemble and standard, single-tree approaches. Accordingly, all results reported here were generated using this parameter setting.

Table 2 summarizes predictions from LOO experiments involving the ensemble and standard RP techniques. Results from multiple decision trees are seen to be significantly more accurate, particularly in the case of inhibitors. Overall, the ensemble approach is correct 75% of the time, compared to only 62% for the standard RP technique. The lackluster performance of the latter may be somewhat surprising, particularly if expectations are based on the ability of each tree to separate its own 99-member training set into inhibitors and noninhibitors. Trees from the standard RP runs typically classify more than 85% of the training set compounds correctly, which is a far cry from the 62% accuracy rate observed for the LOO predictions. This also demonstrates the extreme sensitivity of standard RP to changes in the training set. If decision trees were truly unaffected by removal of a single compound, then one would expect the LOO predictions to be close to 85% accurate.

Figures 3 and 4 illustrate the convergence of ensemble LOO predictions for various compounds as the number of

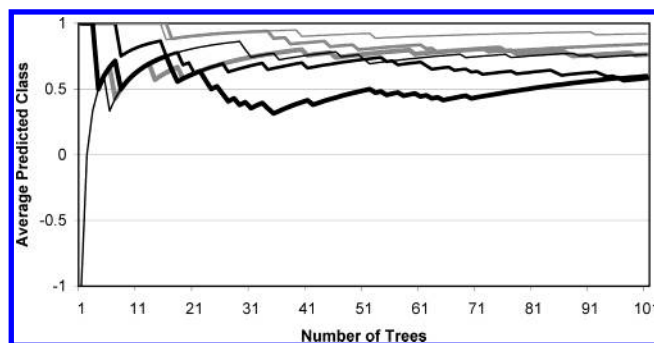


Figure 3. Convergence of ensemble LOO predictions for selected inhibitors.

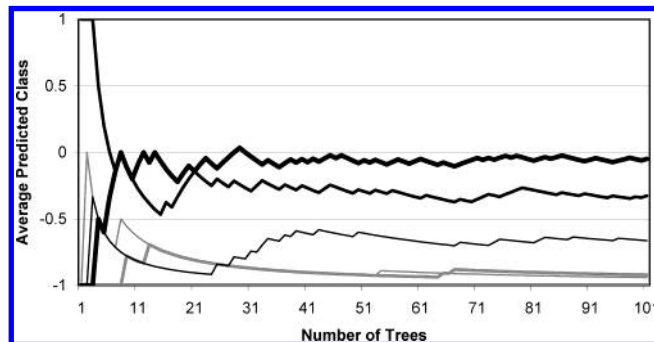


Figure 4. Convergence of ensemble LOO predictions for selected noninhibitors.

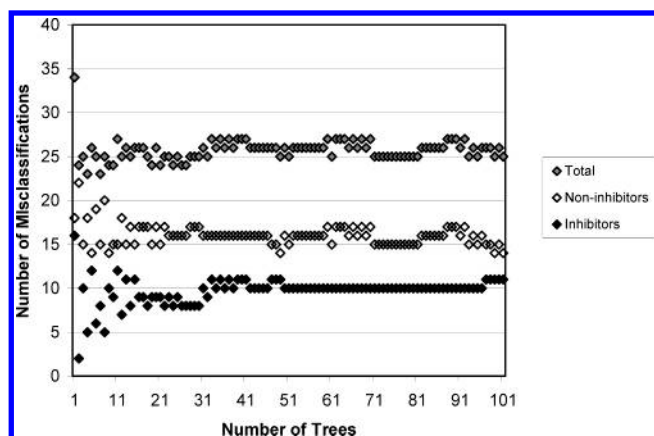


Figure 5. Convergence of misclassification totals for ensemble LOO predictions.

trees is increased from 1 to 101. Average class values ultimately flatten and exhibit no appreciable drift away from the identified algebraic category. Even the upper-most noninhibitor of Figure 4, which hovers just below the 0.0 line, shows no signs of crossing into the inhibitor (+) region. Note, however, that some class values near the border do oscillate back and forth, as evidenced by the overall misclassification rates in Figure 5. Even after 101 trees have been generated, there are still one or two compounds that move between the + and - categories.

Figure 6 demonstrates the advantage of providing classifications with confidence levels. Here, the accuracy of the ensemble LOO predictions is plotted as a function of the average class value. With the exception of the spike in the [0.0, 0.2] interval, which contains only two compounds, a near U-shaped pattern results. Of the 34 predictions that fall into the [-0.4, +0.4] range, only 20 are correct (59%). By contrast, 55 of 66 predictions (83%) with confidence levels

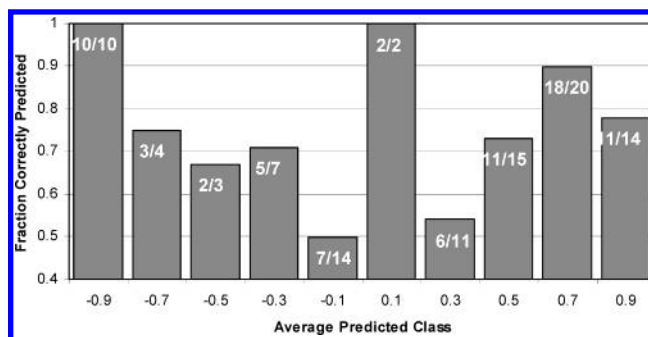


Figure 6. Accuracy rates of ensemble LOO class predictions as a function of average predicted class value. All compounds to the left of 0.0 are predicted to be noninhibitors, whereas all compounds to the right of 0.0 are predicted to be inhibitors. Numbers of correct classifications are indicated within each interval.

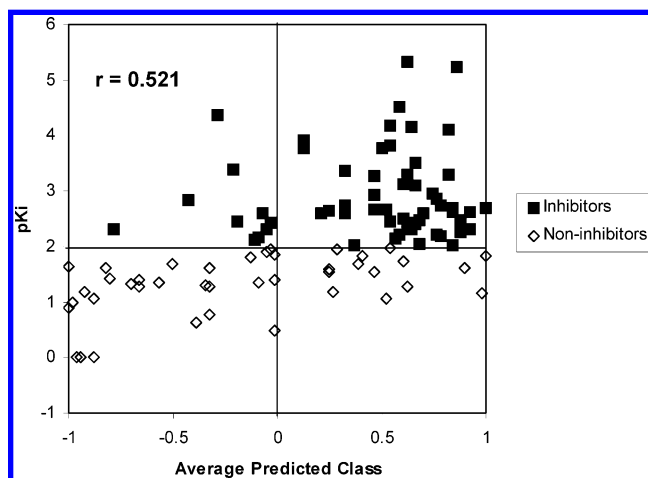


Figure 7. Scatter plot showing the relationship between observed inhibition constants (pK_i) and average predicted class for ensemble LOO validations.

to the left and right of this range are correct. This suggests the confidence level does in fact provide an indication of the reliability of the prediction.

Another interesting characteristic of the ensemble LOO predictions is shown in Figure 7, where observed pK_i values are plotted against average predicted class. Despite the fact that all trees were generated with reference to only a binary activity variable, the average class values do in fact show some relationship to the underlying pK_i data. These data correlate at 0.521, which is somewhat stronger than the 0.482 correlation observed between pK_i and predicted binary class values (± 1).

Obviously, the characteristics and performance of the models are dependent upon the choice of a 10- μ M activity threshold. In general, if the K_i cutoff is raised to include weaker inhibitors in the active subset, the rate of false positive predictions increases. This is simply because weak inhibitors tend to exhibit more structural diversity than strong inhibitors, so the models must be trained to recognize broader classes of compounds as potential inhibitors. Although raising the K_i cutoff should rightly bring about an increase in the number of positive predictions, the rate of increase normally exceeds what is needed to simply encompass the naturally broader population of inhibitors.

One might argue that the cutoff should be assigned to yield models that exhibit equivalent accuracy with respect to

Table 3. Summary of External Predictions

RP method	correct classifications		
	inhibitors	noninhibitors	all
ensemble	10/10 (100%)	31/41 (76%)	41/51 (80%)
standard	10/10 (100%)	26/41 (63%)	36/51 (71%)

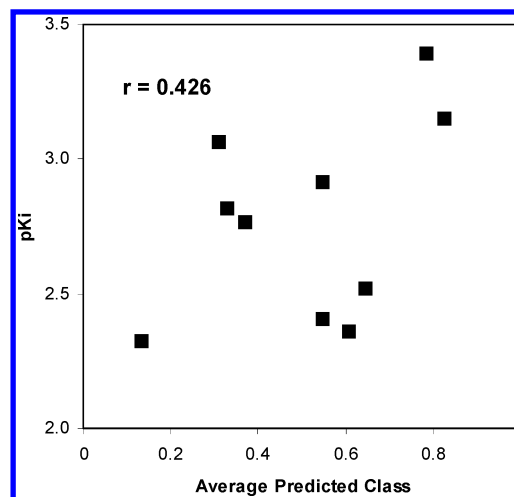
inhibitors and noninhibitors. In the present case, this would require the K_i threshold to be lowered to some value smaller than 10 μ M. Note, however, that the model would then be trained to classify as noninhibitors a number of compounds that could be exerting a significant biological effect. This is clearly undesirable if the goal is to prevent such compounds from ever being pursued as drug candidates.

A more general solution to the threshold problem is to use more than two activity categories. We have investigated this approach, using three levels of activity, but found that a surprisingly small fraction of compounds are actually classified into the correct category. Many of these misclassifications are off by only one level of activity, so one might score these as partial misclassifications. Nevertheless, this sort of approach is no more informative than using a binary activity variable and predictions with confidence levels.

Results presented thus far were derived from LOO predictions and therefore reflect the performance of 100 different models built from 100 different training sets containing 99 compounds. As noted before, these tests are an important validation of the methodology itself, but, ultimately, a single, definitive model should be constructed and applied to an independent set of compounds. Accordingly, the entire 100-member training set was utilized to construct a final ensemble RP model and a final standard RP model. As before, a total of 101 trees were generated for the ensemble approach, using random samples containing 35 inhibitors and 35 noninhibitors. Ensemble and standard RP models were then used to classify the 51 external validation compounds.

As shown in Table 3, both types of models correctly identify all 10 inhibitors, but the ensemble approach is more accurate with respect to noninhibitors, and, therefore, more accurate overall. Internal and external validations both suggest a greater sensitivity toward identifying inhibitors, implying a comparatively low false-negative rate. It follows that compounds classified as noninhibitors can be expected to have a distinctly lower-than-average probability of causing drug–drug interactions through the CYP2D6 pathway. This characteristic is advantageous when designing ADME filters, as these compounds can be pursued with a significantly lower risk of encountering drug–drug interactions.

Average class values for the external predictions are also observed to correlate, albeit weakly, with pK_i , as illustrated in Figure 8. Here, only inhibitors are included because K_i values for the noninhibitors were generally not reported on a continuous, quantitative scale. Although the correlation is not overwhelming ($r = 0.426$), there is a fairly discernible relationship. This obviously does not imply that the present approach is capable of yielding reliable, quantitative predictions of pK_i , but it does indicate that the confidence levels are meaningful. Indeed, there would be absolutely no correlation if binary predictions were used because all class values would be $+1.0$.

**Figure 8.** Scatter plot showing the relationship between observed inhibition constants (pK_i) and average predicted class for inhibitors in the external validation set.

CONCLUSIONS

Increasing awareness of the massive costs of ADME/Tox failures has resulted in a shift in the drug discovery paradigm toward early elimination of problematic compounds. Development of reliable in silico ADME/Tox filters poses tremendous challenges due to the diversity of the chemical space to which these filters will be applied. Compounding this problem is the shortage of consistent experimental data in the literature. A new generation of predictive methodologies is clearly required for success in this increasingly important field.

Previous work¹² has shown that an ensemble approach to recursive partitioning (RP) provides highly robust activity predictions against a variety of targets, using different families of chemical descriptors. The ensemble RP technique has been applied here to identify inhibitors of cytochrome P450 2D6 (CYP2D6), an enzyme that catalyzes the oxidation of numerous important therapeutic agents. While consistent quantitative inhibition data are not particularly abundant for this enzyme, a relatively small yet diverse training set was assembled and used to construct an ensemble RP model of CYP2D6 inhibition. This model utilizes 2D structural descriptors to classify a compound as either inhibitor or noninhibitor and assigns a confidence level to that prediction. Internal and external validation tests indicate that correct classifications may be expected 75–80% of the time. Further, the confidence level can be used to identify the most reliable predictions, pushing the accuracy beyond 80%. Results reported here indicate that this new generation of modeling tools holds great promise in the challenging field of ADME/Tox prediction.

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