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INTRODUCTION

Early consideration of ADME (absorption, distribution, metabolism, and excretion) properties is increasingly seen as essential for efficient discovery and development of new drugs and drug candidates. Cytochromes P450 (CYPs) constitute the most important family of biotransformation enzymes involved in drug metabolism. Metabolism determines the fate of a compound entering the body and plays an important role in the disposition of drugs and their pharmacological and toxicological effects. When one compound inhibits CYPs, the subsequent decrease of oxidative metabolism of another compound can lead to unexpected side effects/toxicity due to accumulation of the latter compound being not metabolized. Therefore, inhibition of CYPs is detrimental for a potential drug candidate.

Cytochrome CYP2D6 is a polymorphic member of the P450 super-family and is one of the major responsible of the oxidative metabolism of current drugs. Developing *in silico* filters able to identify probable inhibitors of CYP2D6 is particularly attractive as they may be applied to entire chemical libraries at the outset of the drug discovery. We describe here the generation of different QSAR models able to predict CYP2D6 inhibition based on different sets of descriptors: DRAGON and EVA descriptors. As an independent approach, the program PASS - Prediction of Activity Spectra for Substances - (V. Porokhov, D. Filimonov & Associates; www.lbmh.msk.su/PASS/) that can predict several hundreds biological activity probability values, such as pharmacological effects, mechanisms of action, toxicity and metabolism reactions, was trained to predict the probability of CYP2D6 inhibition. Although different approaches have similar prediction accuracies, they do not predict all the same compounds correctly. There is a large degree of overlap between different predictions, but there are compounds that are accurately classified by one method and not the other. This difference enables us to combine predictions in a consensus score able to enhance results obtained with a single prediction scheme.

COMPUTATIONAL APPROACHES AND RESULTS

DATA SET

The dataset is formed by 715 in-house compounds with experimental P450 2D6 IC_{50} values. For 577 compounds an explicit IC_{50} values is available, for the remaining 138 compounds just a qualitative experimental value is available. The 577 compounds were divided into a **Training set** and a **Test set 1** respectively formed by 145 and 432 compounds selected by means of a Onion/D-Optimal Design using the software Mode1 [1]. The generated models were also employed to classify molecules as active or inactive, considering 6.0 as threshold value of pIC_{50} , predicted or experimental and 0.3 as threshold value of PASS pass predicted values. A new test set was considered, **Test set 2**, that contains 570 compounds, formed by structures of test set 1 and the remaining compounds with no explicit experimental IC_{50} value. Test set 2 was employed to evaluate the quality of prediction models, evaluating the fraction of compound well classified as active or inactive together with training set and test set 1.

PASS Predictions

PASS (Prediction of Activity Spectra for Substances) [2] predicts the probability for any given compound to be active (P_a) or inactive (P_i) for each one of over 1000 biological activities, including pharmacological effects, mechanisms of action, mutagenicity, carcinogenicity, teratogenicity, and embryotoxicity. P_a and P_i values vary from 0 to 1, and their sum may be different than 1. PASS predictions are based on the analysis of structure-activity relationships for a training set including a great number of non-congeneric compounds with different biological activities, using the descriptor Multiview Neighborhoods of Atoms (MNA). PASS training set consists of over 46,000 biologically active compounds; 16,000 are already launched drugs and 30,000 drug-candidates under clinical or advanced preclinical testing.

The program PASS was trained to predict the probability of P450 2D6 inhibition, using a set of molecules with pIC_{50} values greater than or equal to 6.0. In this study we choose a PASS probability value (pa) of 0.3.

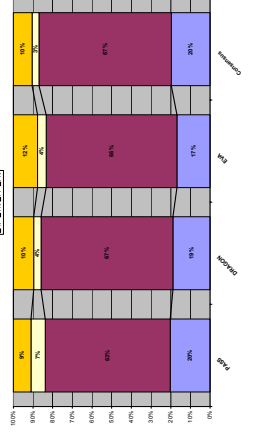
Final Results

To evaluate the quality of our prediction models, we have analysed the fraction of compounds well classified according to predicted value of pIC_{50} , in terms of TP (true positive) FP (false positive), TN (true negative), FN (false negative), accuracy or specificity, coverage or selectivity and Matthews correlation coefficient (MCC) [3]. A perfect prediction gives a correlation coefficient MCC of 1.

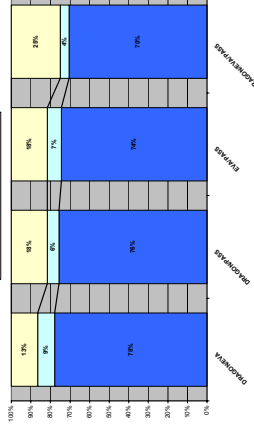
The overall quality of prediction of any single model is quite similar. PASS predicts more FP than others models, and gives the best results in terms of FN, while EVA and DRAGON give a larger number of FN than FP. The Consensus criterion assigns the activity class according to at least 2 of the 3 prediction available, and therefore results more accurate than classification made according to any other single QSAR model.

We have also considered a different kind of classification scheme, looking for a consensus between two or three prediction models at the same time, and classify the molecules as "not predictable" (NP) in any other case. In this way we have obtained a slightly worse results in terms of fraction of molecules predicted correctly, but the risk of FP or FN are reduced, especially for the DRAGON/EVA/PASS consensus, as results clearly from the value of MCC.

Prediction results for the entire data set: 715 compounds



Consensus results for the entire data set: 715 compounds



Results for the entire data set: 715 compounds

Descriptor	TP	TN	FP	FN	accuracy	selectivity	MCC	NP
PASS	145	454	52	64	74	69	0.60	69
DRAGON	135	479	27	74	83	65	0.58	68
EVA	121	475	31	88	80	58	0.58	131
Consensus	141	481	25	68	85	67	0.67	131
DRAGON/EVA	98	459	11	51	90	66	0.71	96
DRAGON/PASS	102	439	12	31	89	77	0.78	131
EVA/PASS	95	437	14	38	87	71	0.74	131
DRAGON/EVA/PASS	77	427	6	26	93	75	0.80	179

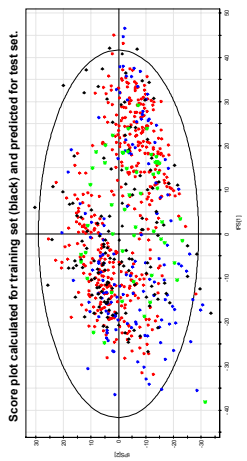
DRAGON and EVA QSAR Models

Dragon is a software package for the calculation of molecular descriptors by Milano Chemometrics and QSAR Research Group. It allows calculation of more than 1600 molecular descriptors for thousands of molecules [3].

EVA, A descriptor of molecular structure based on theoretically derived normal coordinate EigenValue (EVA) [4], related to IR spectra and then strictly dependent on nature and size of neighboring atoms. The normal modes of vibration are calculated using the software Spartan [5]. Calculation of EVA descriptor from vibrational frequencies was carried out using the proprietary program EVA-Q2 (S-IN).

PLS modeling has been used to investigate likely correlations between EVA and experimental pIC_{50} values and, respectively, descriptors generated by DRAGON and experimental pIC_{50} values. The optimal number of components in each PLS model was determined by SIMCA-P+ default cross-validation procedure. Different approaches were explored in order to obtain the best models in terms of stability and predictivity: (a) variables selection carried out on the basis of VIP parameter and coefficient values and (b) SIMCA-P+ Orthogonal Signal Correction (OSC) algorithm [1], used to remove from X data matrices information that is orthogonal to Y.

We obtained a SDEP value of about 0.6 log unit in predicting P450 2D6 inhibition value for both DRAGON and EVA models employing Test set 1, an external test set formed by 432 compounds.



PCs	Obj	R2	O2	SDEC	SDEP	
DRAGON	2	145	0.94	0.93	0.768	0.5824
EVA	1	145	0.74	0.67	0.3888	0.6384

CONCLUSIONS

The availability of different and independent methods and models able to predict P450 2D6 inhibition allow the application of a consensus criterion to be used as a filter in the discovery process. Two QSAR models were obtained with O2 values ranging from 0.87 to 0.93 and SDEP values ranging from 0.63 to 0.56. Employing together PASS and QSAR predictions, we obtained a consensus criterion that applied to 715 molecules yields a Matthews correlation of MCC = 0.67.

The use of a new classification scheme where a prediction is only given when different models agree reduce the risk of wrong classification and introduce a new activity class called here NP (not predictable), that are compounds whose classification is uncertain and requires a deeper analysis. With this classification scheme we obtain a MCC ranging from 0.71 to 0.80.

Considering that, our models might be a powerful *in silico* screen for drug discovery process.

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