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The Development, Validation, and Use of Quantitative Structure Activity Relationship Models of 5-Hydroxytryptamine (2B) Receptor Ligands to Identify Novel Receptor Binders and Putative Valvulopathic Compounds among Common Drugs

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Abstract

Some antipsychotic drugs are known to cause valvular heart disease by activating serotonin 5-HT_{2B} receptors. We have developed and validated binary classification QSAR models capable of predicting potential 5-HT_{2B} binders. The classification accuracies of the models to discriminate 5-HT_{2B} actives from the inactives were as high as 80% for the external test set. These models were used to screen *in silico* 59,000 compounds included in the World Drug Index and 122 compounds were predicted as actives with high confidence. Ten of them were tested in radioligand binding assays and nine were found active suggesting a success rate of 90%. All validated binders were then tested in functional assays and one compound was identified as a true 5-HT_{2B} agonist. We suggest that the QSAR models developed in this study could be used as reliable predictors to flag drug candidates that are likely to cause valvulopathy.

Introduction

During the last decade, several drugs have been shown to cause cardiac valvulopathy in humans. The initial discovery of drug-induced valvulopathy occurred when the anorectic drug fenfluramine (approved by the FDA in 1973), one of the active ingredients of the anorectic drug combination fen-phen, was found to increase the risk of developing two potentially serious conditions, pulmonary hypertension and valvular heart disease (VHD), in individuals receiving these medications to treat obesity.[1] More recently, a group at the Mayo Clinic reported VHD in patients taking the anti-Parkinson drug pergolide.[2] After the initial 2002 report, other cases of VHD associated with pergolide or other dopamine agonists such as cabergoline used as anti-parkinsonian drugs were identified.[3-5] In January of 2007, the New England Journal of Medicine published two large European studies that independently verified the association of VHD with pergolide and cabergoline.[6;7] Finally, on March 29, 2007, the Food and Drug Administration issued a Public Health Advisory for the voluntary market withdrawal of pergolide. These stunning withdrawals of drugs from the market

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Supporting Information Available: 5-HT_{2B} datasets and external validation sets, virtual screening hits, and further computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

stressed the importance of elucidating the mechanism by which these drugs induce valvulopathy and of determining the valvulopathic risk that may be associated with new drug candidates or even existing drugs.

To date, all but two of the VHD-associated drugs are ergoline derivatives (dihydroergotamine, methysergide, pergolide and carbergoline) (see Table 1). The two non-ergoline VHD-associated drugs are fenfluramine[1] and 3,4-methylenedioxymethamphetamine (MDMA, ecstasy),[8;9] both of which are amphetamine analogues (see Table 1). Thus, it appears that compounds from both the ergoline and phenylisopropylamine families can produce VHD.[10]

There is increasing evidence that activation of serotonin 2B receptors (5-HT_{2B}) may play a significant role in the pathogenesis of drug-induced valvulopathy.[11-13] For instance, VHD-associated drugs such as fenfluramine,[14] ergotamine,[14] pergolide[9;15] and cabergoline, and/or selected active metabolites (such as norfenfluramine and methylergonovine),[14] all potently activate 5-HT_{2B} receptors. Chemically similar medications that do not activate 5-HT_{2B} receptors (e.g., lisuride) seemingly do not cause valvular heart disease, further implicating the 5-HT_{2B} receptor—but not other receptors that bind ergopeptines/ergolines and phenylisoproylamines with high affinity—in the pathogenesis of heart-valve disease.[13]

Additionally, valvulopathy-associated drugs have been shown to induce DNA synthesis in cultured interstitial cells from human cardiac valves via 5-HT_{2B} receptor activation.[9] It has been suggested that the valvulopathy induced by 5-HT_{2B} receptor agonists is caused by the inappropriate mitogenic stimulation of normally quiescent valve cells, resulting in an overgrowth valvulopathy.[9;13] Although the precise signaling pathways underlying drug-induced valvulopathy remain elusive, 5-HT_{2B} receptors are known to activate mitogenic pathways through the phosphorylation of Src kinase and extracellular regulated kinases (ERK), as well as through receptor tyrosine kinase transactivation,[16;17] consistent with a role in regulating heart valve interstitial cell proliferation.

The discoveries that 5-HT_{2B} receptors were (1) abundantly expressed in heart valves,[18] (2) activated by fenfluramine and its metabolite, norfenfluramine,[11;18] and (3) activated by other valvulopathy-inducing drugs[9;11] suggested that 5-HT_{2B} receptors were involved in the etiology of valvulopathy.[11;18] Subsequently, several other 5-HT_{2B} agonists were also found to be valvulopathogenic.[9] Since 5-HT_{2B} agonists have the potential of causing valvulopathic side-effects, it has been suggested that all pharmaceuticals should be screened for activity at 5-HT_{2B} receptors prior to further commercial development.[13;19]

Similar to experimental high throughput screening (HTS), virtual screening (VS) is typically employed as a ‘hit’ identification tool.[20] The experimental screening of all molecules against all biological targets is generally cost- and time-prohibitive. Therefore, pre-selection of compounds by VS that have a reasonable probability to act against a given biological target is highly attractive. Typically, VS approaches imply the use of structure based methodologies; nevertheless, we have repeatedly advocated for the use of ligand based cheminformatics approaches such as QSAR models in virtual screening (reviewed in a recent monograph[21]).

Herein, we report on the development of *in silico* screening tools for identifying compounds with potentially serious valvulopathic side effects. These tools can be employed as filters to flag and de-select the potentially harmful compounds at the preclinical stage of drug development, thereby potentially avoiding significant economic and human health consequences incurred at later stages of drug discovery. To achieve this goal, validated and externally predictive, binary QSAR models were generated for 5-HT_{2B} active *vs.* inactive compounds as defined in 5-HT_{2B} functional assays. Similar studies to develop QSAR

models for 5-HT_{2B} actives *vs.* inactives were reported recently by Chekmarev *et al*.[22] However, in our investigations we considered a larger dataset that contained the most complete set of all known valvulopathogens reported by Huang *et al*,[23] and we validated our predictions experimentally in binding assays.

To obtain the most statistically robust and predictive models, we have employed the combinatorial QSAR strategy[24;25] implemented as part of our predictive QSAR modeling workflow (reviewed in Tropsha and Golbraikh[26]). All models were subjected to rigorous internal and external validation. The results confirmed the high external prediction accuracy of our computational models, which led us to conclude that these models can be used reliably to screen chemical databases to identify putative 5-HT_{2B} actives. Screening the World Drug Index (WDI) database using these models led to the identification of 122 possible 5-HT_{2B} actives; 10 of these computational hit compounds were experimentally tested in 5-HT_{2B} radioligand binding assays at the NIMH Psychoactive Drug Screening Program (PDSP), UNC Chapel Hill (http://pdsp.med.unc.edu/). Experiments confirmed that 9 out of 10 compounds were true actives implying a hit rate of 90%. These results indicate the reliability of our computational models as efficient predictors of compounds' affinity towards 5-HT_{2B} receptors. We suggest that the computational models developed in this study could be used as drug liability predictors similar to commonly used predictors[27;28] of other undesired side effects such as carcinogenicity,[29-31] mutagenicity,[29;32;33] PGP binding,[24] or hERG binding.[34-37] Our models can be used to flag compounds that are expected to bind to 5-HT_{2B} receptors but they cannot distinguish agonists from antagonists. Nevertheless, as demonstrated in this study, these putative 5-HT_{2B} binders can be tested in functional assays for their potential to activate 5-HT_{2B} receptors to further assess their valvulopathic potential.

Materials and Methods

Dataset

The PDSP recently screened roughly 2,200 FDA-approved drugs and investigational, drug-like molecules against 5-HT_{2B} receptors.[23] However, this modeling study was initiated prior to the completion of the screening of the entire compound library. At the time this study began, screening against 5-HT_{2B} receptors had been completed for 800 compounds. This set became the basis for our model development. After preprocessing of the 800-compound dataset and deleting duplicates, the final dataset consisted of a class of 146 'actives', and another class of 608 'inactives'. Detailed PDSP protocols are available online (http://pdsp.med.unc.edu/) and in Huang *et al*.[23] All chemical structures were obtained from PubChem[38] as SDF files. By the time our modeling studies were completed, functional data for the remainder of the 2,200 compounds (1,400 compounds) had become available. These 'new' data became a source for additional, independent validation sets.

Preprocessing of the Dataset

For the purposes of this work, the data were curated as follows: First, all molecules were "washed" using the Wash Molecules tool in MOE[39] (v.2007.09). Using this tool, we processed chemical structures by carrying out several standard operations including 2D depiction layout, hydrogen correction, salt and solvent removal, chirality and bond type normalization (all details are found in the MOE manual[39]). Second, we used ChemAxon Standardizer[40] to harmonize the representation of aromatic rings. Finally, the analysis of the normalized molecular structures resulted in detection of 46 duplicate compounds (i.e., different salts or isomeric states). The functional data for duplicated compounds were found to be identical, so in each case a single example was removed. The curated subset of the original 5-HT_{2B} dataset used in this work contains 754 unique organic compounds (146

actives and 608 inactives). All details about the dataset are available in Supporting Information.

Dataset Division for Model Building and Validation

All QSAR models generated in this study to classify actives *vs.* inactives were validated by predicting two external validation sets. Each dataset employed in QSAR studies was first randomly divided into a modeling and a validation sets. Additionally, as described above, an independent validation set became available after we completed our modeling studies. Details about this external set are available in Supporting Information, and in Huang *et al*.[23]

Another level of internal validation was achieved by comparing model performance for training and test sets. This approach is always employed as a part of our predictive QSAR modeling workflow[26] to emphasize the fact that training-set-only modeling is not sufficient to obtain reliable models that are externally predictive.[41] Thus, for each collection of descriptors, the modeling sets were further partitioned into multiple chemically diverse training and test sets of different sizes using the Sphere Exclusion method implemented in our laboratory.[42] Only models that were highly predictive on the test sets were retained for the consensus prediction of the external validation sets. Finally, only those models that were shown to be highly predictive on both external sets were used in consensus fashion for virtual screening of external compound libraries.

Computational Methods

A combinatorial QSAR approach (Combi-QSAR)[24;25] was used to generate classification models for actives *vs.* inactives (Fig. 1). In this study, four types of descriptors were applied in combination with three types of statistical methods.

Molecular Descriptors

Four sets of molecular descriptors were considered in our modeling studies: Dragon,[43] MolConnZ (MZ),[44] MOE,[39] and subgraph descriptors (SG)[45] developed in this laboratory. Each type of descriptors was used separately with each of the classification methods in the context of our Combi-QSAR strategy.

DRAGON Descriptors—The Dragon Professional version 5.4 software[43] was used to calculate 2D descriptors. These included topological descriptors, constitutional descriptors, walk and path counts, connectivity indices, information indices, 2D autocorrelations, edge adjacency indices, Burden eigenvalues, topological charge indices, eigenvalue-based indices, functional group counts, atom-centered fragments and molecular properties. The initial descriptor set was reduced by eliminating the constant and near-constant variables using built-in functions within the software. The pairwise correlations for all descriptors were examined and one of the two descriptors with the correlation coefficient R^2 of 0.95 or higher was excluded. The calculation procedures for these descriptors, with related literature references, are reported by Todeschini and Consonni.[46] Finally, the remaining descriptors were normalized by range-scaling so that their values were distributed within the interval 0-1.

MolConnZ Descriptors—The MolConnZ software[44] available from EduSoft affords the computation of a wide range of topological indices of molecular structure. These indices include, but are not limited to, the following descriptors: valence, path, cluster, path/cluster and chain molecular connectivity indices,[47-49] kappa molecular shape indices,[50;51] topological[52] and electrotopological state indices,[53-56] differential connectivity indices,[47;57] graph's radius and diameter,[58] Wiener[59] and Platt[60] indices, Shannon [61] and Bonchev-

Trinajstić[62] information indices, counts of different vertices, counts of paths and edges between different types of vertices (http://www.edusoftlc.com/molconn/manuals/400). Descriptors with zero values or zero variance were removed; the remaining descriptors were normalized by range-scaling so that their values were distributed within the interval 0-1.

MOE Descriptors—MOE 2007.09 software[39] was used to generate 2D MOE descriptors. These included physical properties, subdivided surface areas, atom and bond counts, Kier and Hall connectivity[47-49] and kappa shape indices,[50;51] adjacency and distance matrix descriptors,[58;59;63;64] pharmacophore feature descriptors, and partial charge descriptors. [39] Descriptors with zero values or zero variance were removed; the remaining descriptors were normalized by range-scaling so that their values were distributed within the interval 0-1.

Subgraph Descriptors (SG)—Frequent subgraph mining of chemical structures is a novel approach to generating fragment descriptors that was developed recently in our group.[45] SG descriptors are derived from each dataset, i.e., not pre-defined which gives the advantage of finding important chemical fragments that may have not been defined *a priori* by other fragment descriptor generating methods. The fragments are derived based on recurring substructures found in at least a subset of molecules (defined by a support value σ) in the dataset. These recurring substructures can implicate chemical features responsible for compounds' biological activities. First, chemical structures were converted into labeled, undirected graph representations where nodes were labeled by atom types and edges corresponded to chemical bonds. Fast frequent subgraph mining (FFSM) algorithm[65] was then used to find common frequent subgraphs for a given support value (σ), which is one of the variables defined by the user that determines the size of the set of subgraphs generated using FFSM. Obviously, the larger is the value of the support, the smaller is the number of subgraphs descriptors. As the support value decreases, the number of subgraphs increases dramatically. Redundant subgraphs were identified and removed leaving only the so called "closed subgraphs". A subgraph *SGi* is closed in a database if there exists no supergraph *SGj* such that $SGi \subseteq SGj$ and $\sigma_{SGi} = \sigma_{SGj}$. However, subgraph *SGi* would not be deleted if it also occurs by itself (not as part of the *SGj*) in the graph database. Removing redundant subgraphs (fragments) reduces the number of subgraphs descriptors drastically and therefore makes the subsequent calculations more efficient. The frequency of individual 'closed subgraphs' in each molecule of the dataset is calculated and used as the descriptor value for each molecule. In this study, a support value of 12 % was used, and the upper size limit of the generated subgraphs was 7 atoms.

Balancing the Dataset Using Similarity Searching

The dataset used for model building was imbalanced, consisting of 146 actives *vs.* 608 inactives. Therefore, only a subset of the larger class of inactives of approximately the same size as the actives was used in model building unless otherwise indicated. This subset was selected to include inactives that were most similar to the actives. Given the vast array of available chemical descriptors and the large number of similarity measures, it is always difficult to decide *a priori* which combination of descriptors/similarity metrics to use. This problem has been highlighted in several recent publications.[66;67] Therefore, similarity searching studies were performed using three types of molecular descriptors: fingerprints (FP), Dragon, and MZ, and applying two similarity metrics, i.e., Euclidean distance and Tanimoto coefficient (Tc). The similarity cutoff was chosen to obtain the most balanced (with roughly equal number of compounds from each class) subset of compounds.

Fingerprints (FP)—166 MACCS[68] structural keys implemented in MOE 2007.09 software[39] were calculated for all compounds. The similarity searching was performed using an in-house written script applying Tanimoto coefficients for similarity measures.

Dragon Descriptors—Normalized Dragon descriptors of the original dataset were employed to calculate similarities between all compounds in the dataset using Euclidean distance as similarity metric; variable similarity thresholds were used to down-sample the larger class (inactives). Although many schemes could be considered for down-sampling the larger classes, we used the similarity threshold based approach since it restricts the larger class to compounds most similar to the smaller class molecules. This approach makes it more challenging to develop statistically significant models capable of discriminating smaller class compounds from most chemically similar molecules in the larger class. Therefore, it increases the robustness of the binary QSAR models.

MolConnZ Descriptors (MZ)—Similar procedures to those described above for Dragon descriptors were used.

QSAR Methods

***k* Nearest Neighbors (*k*NN) QSAR—**The *k*NN QSAR method[69] is based on the *k* nearest neighbors principle and the variable selection procedure. It employs the leave-one-out (LOO) cross-validation (CV) procedure and a simulated-annealing algorithm[70;71] to optimize variable selection. The procedure starts with the random selection of a predefined number of descriptors from all descriptors. If the number of nearest neighbors *k* is higher than one, the estimated activities $\hat{y}_i$ of compounds excluded by the LOO procedure are calculated using the following formula:

$$\widehat{y_i}=\frac{\sum_{j=1}^{k} y_j w_{ij}}{\sum_{j=1}^{k} w_{ij}} \quad (1)$$

where y_j is the activity of the *j*-th compound. Weights w_{ij} are defined as:

$$w_{ij}=\left(1+\frac{d_{ij}}{\sum_{j'=1}^{k} d_{ij'}}\right)^{-1} \quad (2)$$

and d_{ij} is Euclidean distances between compound *i* and its *j*-th nearest neighbor. However, if the number of nearest neighbors *k* is equal to one, then the estimated activity $\hat{y}_i$ of the compound will be equal to the activity of this one nearest neighbor.

For classification *k*NN, the predicted $\hat{y}_i$ values (see expression (1)) are rounded to the closest whole numbers (which are, in fact, the class numbers), and the prediction accuracy (correct classification rate, CCR_{train}) is calculated as follows:

$$CCR=0.5\left(\frac{N_1^{corr}}{N_1^{total}}+\frac{N_2^{corr}}{N_2^{total}}\right) \quad (3)$$

where N_j^{corr} and N_j^{total} are the number of correctly classified and total number of compounds of class *j* (*j*=1, 2). Then, a predefined small number of descriptors are randomly replaced by other descriptors from the original pool, and the new value of CCR_{train} is obtained. If CCR_{train} (new) > CCR_{train} (old), the new set of descriptors is accepted. If CCR_{train} (new) ≤ CCR_{train} (old), the new set of descriptors is accepted with probability p = exp (CCR (new) - CCR (old))/T, or rejected with probability (1-p), where T is a simulated annealing (SA)

"temperature" parameter. During this process, T is decreasing until a predefined threshold. Thus, the optimal (highest) CCR_{train} is achieved. For the prediction, the final set of selected descriptors is used, and expressions (1) and (2) are applied to predict activities of compounds of the test sets. Then the activities are rounded to the closest whole numbers, and the correct classification rate for the test set is calculated using formula (3).

In the case when compounds belong to two classes (e.g., active and inactive compounds), a 2 × 2 confusion matrix can be defined, where $N_{(1)}$ and $N_{(0)}$ are the number of compounds in the data set that belongs to classes (1) and (0) respectively. TP, TN, FP, and FN are the number of true positives (actives predicted as actives), true negatives (inactives predicted as inactives), false positives (inactives predicted as actives), and false negatives (actives predicted as inactives), respectively. The following classification accuracy characteristics associated with confusion matrices are widely used in QSAR studies: sensitivity (SE=TP/$N_{(1)}$), specificity (SP=TN/$N_{(0)}$), and enrichment $E = TP*N/[(TP+FP)*N_{(1)}]$. In this study, we have employed normalized confusion matrices. A normalized confusion matrix can be obtained from the non-normalized one by dividing the first column by $N_{(1)}$ and the second column by $N_{(0)}$. Normalized enrichment is defined in the same way as E but is calculated using a normalized confusion matrix: $E_n = 2TP*N_{(0)}/[TP*N_{(0)}+FP*N_{(1)}]$. E_n takes values within the interval of [0, 2].[25;72]

Classification Based on Association (CBA)—This method integrates both classification rule mining,[73;74] which aims to discover a small set of rules in the database that forms an accurate classifier, and association rule mining,[75] which finds all the rules existing in the database that satisfy some minimum support and minimum confidence constraints. For association rule mining, the target of discovery is not pre-determined, while for classification rule mining there is one and only one predetermined target. The integration is done by focusing on mining a special subset of association rules, called *class association rules* (CARs). An efficient algorithm is also used for building a classifier based on the set of discovered CARs.

The CBA algorithm[76;77] consists of two parts, a rule generator, which is based on the *a priori* algorithm for finding association rules, and a classifier builder. The candidate rule generator is similar to the *a priori* one. The difference is that CBA updates the support value in each step while the *a priori* algorithm only updates this value once. This allows us to compute the confidence of the *ruleitem*. A *ruleitem* is of the form: <*condset*, *y*> where *condset* is a set of items, $y \in Y$ is a class label. The support count of the *condset* (called *condsupCount*) is the number of cases in the dataset (D) that contain the *condset*.

Next, a classifier is built from CARs. To produce the best classifier out of the whole set of rules would involve evaluating all the possible subsets of it on the training data and selecting the subset with the right rule sequence that gives the least number of errors. There are 2m such subsets, where m is the number of rules. It is a heuristic algorithm. Given two rules, r_i and r_j, r_i precedes r_j if (1) the confidence of r_i is greater than that of r_j, or (2) their confidences are the same, but the support of r_i is greater than that of r_j, or (3) both the confidences and the supports of r_i and r_j are the same, but r_i is generated earlier than r_j. If R is a set of generated rules (i.e. CARs) and D the training data, the basic idea of the algorithm is to choose a set of high precedence rules in R to cover D. The classifier follows this format: <r_1, r_2, . . ., r_n, default_class>, where $r_i \in R$. In classifying an unseen case, the first rule that satisfies the case will classify it. If there is no rule that applies to the case, it takes on the default class.

The descriptors used with CBA need to be discrete in nature[76] as is the case with SG descriptors but not Dragon, MolConnZ or MOE. Hence, this method was only used with SG descriptors using CBA (v2.1) software.[78]

Distance Weighted Discrimination (DWD)—This method was initially proposed by Marron and Todd[79] with the goal of improving the performance of SVM[80;81] in high dimensional low sample size (HDLSS) contexts. The main idea is to improve upon the criterion used for "separation of classes" in SVM. SVM has data piling problems along the margin, because it is maximizing the minimum distance to the separating plane, and there are many data points that achieve the minimum. A natural improvement is to replace the minimum distance by a criterion that allows all the data to have an influence on the result. DWD does this by maximizing the sum of the inverse distances. This results in directions that are less adversely affected by spurious sampling artifacts. The major contribution of this new discrimination method is that it avoids the data piling problem, to give the anticipated improved generality. Like SVM, DWD is based on computationally intensive optimization; however, while SVM uses well known quadratic programming algorithms, DWD uses interior-point methods for so-called Second-Order Cone Programming (SOCP) problems.[82] Detailed discussion of these issues may be found in Marron and Todd (2007),[79] which is available with the supporting information at https://genome.unc.edu/pubsup/dwd/. All DWD computations were performed using the DWD software[83] written in Matlab[84] and kindly provided by Dr. Marron.

Robustness of QSAR Models

Y-randomization test is a widely used validation technique to ensure the robustness of a QSAR model.[85] This test includes (i) randomly shuffling the dependent-variable vector, Y-vector of training sets (class labels in this study) and (ii) rebuilding models with the randomized activities (class labels) of the training set. All calculations are repeated several times using the original independent-variable matrix. It is expected that the resulting QSAR classification models, built with randomized activities for the training set, should generally have low CCRs for training, test, and external validation sets. It is likely that sometimes, though infrequently, high CCR values may be obtained due to a chance correlation or structural redundancy of the training set. However, if some QSAR classification models obtained in the Y-randomization test have relatively high CCR it implies that an acceptable QSAR classification model cannot be obtained for the given dataset by the particular modeling method. Y-randomization test was applied to all datasets considered in this study, and the test was repeated five times in each case.

Applicability Domain of *k*NN QSAR Models

Formally, a QSAR model can predict the target property for any compound for which chemical descriptors can be calculated. However, since the training set models are developed in *k*NN QSAR modeling by interpolating activities of the nearest neighbor compounds, a special applicability domain (i.e., similarity threshold) should be introduced to avoid making predictions for compounds that differ substantially from the training set molecules.[86]

The similarity was estimated using Euclidean distances in high-dimensional descriptors space. Compounds with the smallest distance between them have the highest similarity. The distribution of distances (pairwise similarities) of compounds in our training set is computed to produce an applicability domain threshold, D_T, calculated as follows:

$$D_T = \bar{y} + Z\sigma \quad (4)$$

Here, $\bar{y}$ is the average Euclidean distance of the k nearest neighbors of each compound within the training set, σ is the standard deviation of these Euclidean distances, and Z is an arbitrary parameter to control the significance level. Based on previous studies, we set the default value of this parameter as 0.5, which formally places the boundary for the applicability domain at one-half of the standard deviation. Thus, if the distance of the external compound from at least one of its nearest neighbors in the training set exceeds this threshold, no prediction is made.[86] In this study two types of applicability domains were employed. First, a global applicability domain that ensures some level of global similarity (using all descriptors for similarity calculations) between the predicted compounds and the compounds in the modeling set. The second is a local domain which is the applicability domain of each of the individual models using only descriptors used for the model building.

Consensus Prediction

Our experience suggests that consensus prediction of the target property for external compounds, i.e., when the compound activity is calculated by averaging values predicted by all individual models that satisfy our acceptability criteria always provides the most stable and accurate solution[87]. In general, consensus prediction implies averaging the predictions for each compound by majority voting for classification QSAR models, using all models passing the validation criteria (e.g., $CCR_{train} \geq 0.70$ and $CCR_{test} \geq 0.70$). In order to determine the confidence in the obtained predictions we need to define a consensus score. The consensus scores employed in this study take into account the total number of models used to predict the compound's activity, and the number of models that predicted the compound to belong to a specific class. Since we define two classes of compounds, i.e., class 1 (actives) and class 0 (inactives), some models may predict a compound to belong to class 0 and others may predict it to belong to class 1. As a result, a consensus score between 0 and 1 will be obtained for each of the predicted compounds. As an additional measure of confidence (and an additional applicability domain criterion) we only accepted those predictions that had an average predicted value (consensus score) above 0.7 (for actives) or below 0.3 (for inactives).

Virtual Screening and Compound Selection for Experimental Validation

To identify putative actives, validated consensus models generated for 5-HT_{2B} ligands were used for virtual screening of ca. 59,000 molecules within the WDI chemical library; the selection of hits was limited by the applicability domains of each models.[88] 122 compounds were identified as VS hits (by consensus agreement between all accepted models, see Table S1 of Supporting Information for details) and 10 structurally diverse and commercially available hits were purchased from different suppliers and tested at PDSP in 5-HT_{2B} radioligand binding assays.

Results and Discussion

Combinatorial QSAR Modeling of 5-HT_{2B} Actives *vs.* Inactives

Balancing the Dataset—The original dataset of 146 actives and 608 inactives was first balanced by downsizing the class of inactives. Similarity searching between active and inactive compounds using Tc cutoff of 0.7 resulted in 195 inactives (that were similar to at least one active compound with Tc above 0.7), which were combined with the 146 actives to form the modeling set of 342 compounds. Dragon and MZ descriptors were generated for this 342-compound modeling set to be used separately with *k*NN. However, similarity searching using Dragon and MZ descriptors and applying Euclidean distance-based threshold resulted in a 304- (146 actives and 158 inactives) and 325-compound (146 actives and 179 inactives) modeling sets respectively. Thus, slightly different modeling sets were used depending on the type of descriptors.

k* NN Classification—k*NN method was used with each of the following descriptor types: DRAGON, MZ, MOE, and SG descriptors. Models were built for the three datasets resulting from the down-sampling of the original dataset. First, a validation set (15-20% of the dataset) was excluded from each of the resulting datasets randomly. The compounds in the remaining modeling set (85-80% of the original dataset) were divided into multiple training and test sets (28-40 divisions). Multiple QSAR models were generated independently for all training sets and applied to the test sets. Generally, we accepted models with CCR values for both the training and test set greater than 0.70. *k*NN combined with subgraphs and Dragon descriptors were the two best performing methods based on validation set statistics (Table 2). *k*NN-subgraphs (*k*NN-SG) had a CCR_{evs} = 0.80, while *k*NN-Dragon gave a CCR_{evs} = 0.72.

Results of the Y-randomization test (Table 2) confirmed that *k*NN classification models with CCR_{train} and CCR_{test} values ≥ 0.70 were robust. None of the models with randomized class labels of the training set compounds had CCR_{rand} > 0.54 for any dataset.

Classification Based on Association (CBA)—The CBA method was applied to classify the dataset using SG descriptors. A dataset of 342 compounds (146 actives and 196 inactives), resulting from the downsizing process with FP and Tanimoto distances, was used. The dataset was split randomly into training (267 compounds) and validation sets (75 compounds). A total of 1371 closed frequent subgraphs were generated with FFSM (see Methods) from the training set using a support value of 12% and a maximum size limit of the fragments of 7. The training set consisting of 267 compounds (111 actives and 156 inactives) was then used to build the classifier in CBA. The classifier gave a CCR_{train} of 0.79. Then the validation set consisting of 75 compounds (35 actives and 40 inactives) was used to assess the robustness of the classifier. The CCR_{evs} was 0.65 which is not as high as the CCR value for the training set.

DWD Modeling—The DWD method was applied to classify the dataset using Dragon descriptors. A dataset of 304 compounds (146 actives and 158 inactives), resulting from the downsizing process with Dragon descriptors and Euclidean distances, was used. The dataset was split randomly into training (244 compounds) and validation sets (60 compounds). A total of 387 Dragon descriptors were generated for the training set. The training set consisting of 244 compounds (120 actives and 124 inactives) was then used to build the DWD model. The DWD model was able to group compounds in this dataset based on their biological classes with a CCR_{evs} = 0.70 (TP=18, TN=24, FP=10, FN=8), setting the cutoff at "0.15". DWD was further used to rank Dragon descriptors according to their importance for discriminating the two classes of compounds (actives *vs.* inactives). DWD uses class label information where positive (for actives) and negative (for inactives) signs are assigned to each descriptor value to indicate its importance to the corresponding class. The top 20 highly weighted descriptors (based only on weights' values and ignoring the signs) are presented in Table S2 of Supporting Information.

Comparison of Binary QSAR Approaches for Classifying 5-HT_{2B} Actives *vs.* Inactives

The performance of different binary QSAR approaches employed as part of combinatorial QSAR strategy for 5-HT_{2B}, and based on validation set statistics, is summarized in Figure 2. *k*NNSG, and *k*NN-Dragon were the best performing methods for classifying 5-HT_{2B} actives *vs.* inactives based on validation set statistics (Table 2), yielding the highest CCR_{evs} of 0.80 in case of *k*NN-SG. On the contrary, *k*NN-MZ was the worst performing method with a CCR_{evs} of 0.57 which was very close to random. It was also interesting to see that *k*NN-SG performed much better than CBA-SG with CCR_{evs} = 0.80 in the former case and 0.65 in the

latter. These results confirm the importance of employing the combinatorial QSAR approach to find the most predictive QSAR method/descriptor combination for each specific dataset.

Our models also indicated that the nature of the descriptors used has a dramatic effect on the performance of the modeling methods. It was clear that MOE and MolConnZ descriptors did not perform very well in all tested cases irrespective of the applied modeling techniques. On the contrary, Dragon descriptors afforded most significant models with all methods and in all tests, for both validation and external sets.

Additional Model Validation

Model Validation by Predicting Drugs Known to be 5-HT_{2B} Actives and Valulopathogens—Both fenfluramine and dexfenfluramine (known to be 5-HT_{2B} actives and agonists, which were not included in our modeling sets) were predicted as 5-HT_{2B} actives using consensus models to classify actives *vs.* inactives. The consensus scores using *k*NN-Dragon were 0.79 for both compounds. Our previous studies suggest that consensus prediction that is based on the results obtained by all validated predictive models always provides the most stable solution.[87] A 5-HT_{2B} active compound can have consensus scores in the interval [0.5-1.0]. The closer value to 1.0 the greater is the confidence in the prediction. Therefore, we can claim that both compounds were predicted as actives with statistically significant consensus scores.

These results highlight the predictive power of our validated models that could have predicted the possible dangerous side effects of these two drugs by suggesting that they may be 5-HT_{2B} actives. This prediction would have suggested that these compounds should be tested experimentally in 5-HT_{2B} functional assays and prevented from further development as potentially unsafe medicines. This example illustrates the potential use of models developed in this study as computational drug safety alerts.

Model Validation by Predicting an External Set—An additional 16-compound set was obtained from PDSP after we finished out modeling studies. This external set was used to further assess the robustness and the predictive power of our models. All 16 compounds were 5-HT_{2B} actives including 4 agonists and 12 antagonists.

The 16 external compounds were predicted using all consensus models built to classify actives *vs.* inactives. *k*NN-Dragon was the best performing method on this external set with a CCR_{ex} of 0.81. Predictions were made by applying local model applicability domains with Z = 0.5 (see Applicability Domain of *k*NN QSAR Models). It was interesting to find that *k*NN-Dragon had CCR ≥ 0.72 with both the validation (CCR_{evs} = 0.72) and the external (CCR_{ex} = 0.81) sets. However, *k*NN-SG (the best performing method on validation sets) was not as good with the external set (CCR_{ex} = 0.65) as it was with the validation set (CCR_{evs} = 0.80). CBA-SG gave a CCR_{ex} = 0.65, which was consistent with its performance with the validation set (CCR_{evs} = 0.65) but less than CCR_{train} (0.79). The latter results using SG descriptors with *k*NN and CBA might be due to the limitation that frequent subgraphs are derived from the training set compounds; therefore, it is possible that fragments that are frequent in the external set are not represented in the frequent subgraphs used for prediction. Our current applicability domain filter, which is calculated using the fragments in the training set, does not account for this possibility. It is clear that a more stringent applicability domain filter could be applied in this case, which uses the distribution of subgraphs counts between the training and test set, but this has not been implemented yet within our current method.

The Importance of Variable Selection

Since *k*NN-Dragon was the best performing method to classify actives *vs.* inactives based on the results for all validation sets, we thought it would be interesting to check the performance of *k*NN using all 387 Dragon descriptors, generated for the actives *vs.* inactives modeling set, without variable selection. The results of this test are shown in Table 3. Comparison of modeling results for *k*NN-variable-selection (CCR_{evs} = 0.72) *vs. k*NN-without-variable-selection (CCR_{evs} = 0.52) clearly indicates that variable selection is a vital part of modeling. Furthermore, the top 20 most frequent descriptors (MFD) selected by *k*NN models (Table S3 of Supporting Information) and top 20 highly weighted descriptors by DWD based only on weights and ignoring the sign (Table S2 of Supporting Information) were used independently with the *k*NN method (with no variable selection) to predict actives *vs.* inactives (Table 3). Models built with either the top 20 DWD-selected Dragon descriptors or MFD from Dragon-*k*NN and using 1-5 nearest neighbors gave $CCR_{evs} \sim 0.5$ (Table 3). These results illustrated again that SA-based variable selection procedures implemented in our *k*NN QSAR method[69] lead to models with the highest external predictive power as compared to any other approach not relying on variable selection for model optimization.

Mechanistic interpretability is frequently regarded as very important feature of QSAR models. We generally argue that only models that have been extensively validated on external datasets and identified experimentally-confirmed hits should be subjected to interpretation. Furthermore, very few classes of models, specifically, those based on (multiple) linear regression and small number of descriptors can afford a relatively straightforward interpretation. The interpretation of multi-parametric statistical models developed with non-linear optimization algorithms (as in this study) should be attempted with great care because of strong and often poorly understood interplay between descriptors. Furthermore, although we could foresee that in some cases medicinal chemists may want to modify their candidate compounds to prevent $5HT_{2B}$ binding, the tools developed in this study are predominantly intended for virtual screening of libraries of drug candidates to flag and possibly eliminate compounds that are likely to bind $5HT_{2B}$ receptor, not to design new compounds; and any compound designed by chemists could be passed through our models. Therefore, we only restricted the discussion in this paper to the most frequent descriptors found by all acceptable *k*NN models and the most highly weighted descriptors selected by DWD to stress that the process of variable selection employed as part of model optimization has indeed converged on a small number of descriptors.

Virtual Screening of the World Drug Index Database to Identify Putative 5-HT_{2B} Ligands

Since our models proved to be reasonably accurate based on two external validation sets, we used the best models to mine a large external database of approved and potential drugs for putative 5-HT_{2B} actives. An important condition that assures reliable predictions by the model is the use of AD. Therefore, two types of AD were employed in the virtual screening of compound databases. The first is a global AD that acts as a filter and ensures some level of global similarity between the predicted compounds and the compounds in the modeling set. The second is a local AD which is defined for each of the individual classification models.

The WDI database of ca. 59,000 compounds (approved or investigational drugs) was used for virtual screening (Fig. 3). This original collection had many duplicates (i.e., many salt forms for the same chemical entity). The duplicates were removed using MOE: keeping unique structures and deleting duplicates. We also removed all compounds included in our modeling and external validation sets. Dragon descriptors were generated for the remaining 46,859 unique compounds in the database; 926 compounds were excluded because Dragon

was unable to calculate at least one of the descriptors generated for the modeling set. The remaining 45,933 compounds were then subjected to a global AD filter for the actives *vs.* inactives modeling set using a strict Z cutoff of 0.5 (which formally places the allowed pairwise distance threshold at the mean of all pairwise distance distribution for the training set plus one-half of the standard deviation). Obviously, increasing the AD would increase the number of computational hits identified by virtual screening. However, our experience suggests that such increase is typically accompanied by the decrease in prediction accuracy. Additionally, we required that the nearest neighbor in the modeling set of a compound from the virtual library be an active. The resulting 7,286 compounds were then classified into actives *vs.* inactives using DWD-Dragon classifier resulting in 891 actives. Next, all *k*NN-Dragon models with CCR_{train} and $CCR_{test} \geq 0.70$ were employed in consensus fashion to predict these 891 compounds resulting in a selection of the 500 active hits. At this point, SG descriptors were generated for these 500 molecules. CBA-SG classifier followed by *k*NN-SG consensus models were used as final filters for the determination of 122 compounds regarded as putative 5-HT_{2B} actives.

Experimental Validation

Ten structurally diverse hits (**1**-**10,** see Table 4) were selected from the final consensus virtual screening hits for further experimental validation taking into account both their commercial availability and cost (see Table 4). To our satisfaction, nine compounds were confirmed to inhibit 5-HT_{2B} radioligand binding, which implies a hit rate of 90 %. K_i values were in the range 0.8 – 3,127 nM, with 4 compounds having K_i values < 100 nM. The four highest affinity compounds were: **4** (K_i=33 nM, see Fig. 4 (A)), **7** (K_i=0.8 nM, see Setola *et al*, 2003[9]), **9** (K_i=70 nM, see Fig. 4 (B)), and **10** (K_i=69 nM, see Fig. 4 (C)).. It should be noted that methylergometrine, though not included initially in our dataset, was known to be a valvulopathic compound and had been tested against 5-HT_{2B} receptors in both binding (K =0.8 nM)[9] and functional assays (pEC_{50} for 5-HT_{2B}-Mediated calcium flux = 7.67)[23]. In order to determine the activity of the remaining eight 5-HT_{2B} ligands, all compounds were tested at the PDSP in 5-HT_{2B} functional assays. Results indicated that methylergometrine was the only compound among the 9 5-HT_{2B} ligands that possessed strong agonist activity.

This low hit rate of 11.1% for identifying validated agonists is in fact not surprising in light of Huang *et al*[23] major finding that potent 5-HT_{2B} receptor agonism is a relatively rare occurrence among drugs and drug-like compounds. However, to arrive at such conclusions, Huang *et al* screened a composite library containing three publicly available collections of FDA-approved and investigational medications and one internally compiled library. Of the approximately 2200 compounds screened, 27 5-HT_{2B} receptor agonists were identified; thus, the validated hit rate was 1.2%.

These results illustrate that the validated QSAR workflow, as employed in this paper, could be used as a general tool for identifying 5-HT_{2B} ligands by the means of virtual screening of chemical libraries using rigorously built QSAR models. As we demonstrated in this study, our models identify a relatively small number of VS hits making it feasible to employ experimental tools to validate predictions in 5-HT_{2B} binding and functional assays. Ten compounds selected from a large external library have been tested experimentally in this proof-of-concept study resulting in very high experimentally confirmed hit rate. The list of all compounds predicted to be 5-HT_{2B} actives is available in the Supporting Information (Table S1).

To verify the diversity of the experimentally validated hits, we have compared the results of QSAR-based virtual screening with simple similarity searches. Similarity calculations were done using two different descriptor-metric combinations: (1) MACCS structural keys and Tanimoto coefficients (as a standard similarity searching approach, see Table S9 and Figure

S1 in Supporting Information) and (2) Dragon descriptors and Euclidean distances (to compare directly with our best performing QSAR models of *k*NN-Dragon, see Table S10 and Figure S2 in Supporting Information). The nearest neighbor compounds (based on Tanimoto similarities and MACCS keys) from the active compounds in the dataset and the 10 experimentally validated VS hits are reported in Table 5. Results of similarity analyses indicated that neither technique would be able to efficiently identify the diverse hits obtained with our methods (see Supporting Information for details). Hence, our studies illustrated the power of combi-QSAR-based VS in prioritizing compounds (which are not just close analogs of the modeling set compounds) from screening libraries to achieve high success rates when experimentally validated.

We also think that agonist *vs.* antagonist models will be highly useful as more data about agonist compounds become available. The small number of known 5-HT_{2B} agonists made it impossible at this stage to develop statistically significant models that could distinguish agonists from antagonists. Thus, the current study was limited to building binder *vs.* non-binder models. We will continue with our efforts to develop quantitative 5-HT_{2B} agonist predictors as we accumulate more experimental data.

Conclusions

QSAR models are becoming increasingly attractive as robust computational tools for virtual screening due to both their computational efficiency and success rates [reviewed in[26] as well as in a recent monograph[21]]. In this study, we have applied a combinatorial QSAR approach to a dataset of 800 compounds experimentally annotated as 5-HT_{2B} receptor agonists, antagonists and inactives resulting in statistically validated and externally predictive models. Specifically, we have applied a combi-QSAR approach utilizing three different classification methods (*k*NN, CBA and DWD) and four different descriptor types (Dragon, MZ, MOE and SGs) to generate classification QSAR models to discriminate between 5-HT_{2B} actives (agonists and antagonists) from inactives. Predictive models with classification accuracies as high as 0.80 for actives *vs.* inactives, as estimated on external validation sets, were obtained.

Classification models for actives *vs.* inactives were further validated by predicting an external validation set obtained after we completed the modeling studies. The high accuracy of prediction for the second external validation set proved that our models were indeed rigorous. Therefore, we posited that our studies afforded a robust computational tool to predict potential 5-HT_{2B} activity and consequently prioritize hits for testing in functional 5-HT_{2B} assays to predict valvulopathic side effects of drugs and drug candidates that act as 5-HT_{2B} agonists. We suggested that this computational predictor could be used to eliminate high risk compounds at the early stages of the drug development process. To illustrate this point, we have used this predictor retrospectively to evaluate the valvulopathic potential of two drugs withdrawn from the U.S. market for this reason, i.e., fenfluramine and dextrofenfluramine. Both drugs were not included in our modeling set and both were indeed predicted with high confidence as actives for binding to 5-HT_{2B} receptors.

Encouraged by our model validation results, we have applied these models for virtual screening of the 59,000 compounds in WDI database. Our classification strategies identified 122 potential 5-HT_{2B} ligands. Ten structurally diverse VS hits were experimentally tested at PDSP. Nine compounds were experimentally confirmed as 5-HT_{2B} ligands thereby demonstrating a very high success rate of 90%.

The predictor developed in this report is similar in its potential use to other predictors of drug liability such as carcinogenicity and mutagenicity that are widely used in pharmaceutical industry. For instance, the TOPKAT program available in the Discovery

Studio,[89] is a QSAR-based system that generates and validates accurate, rapid assessments of various types of chemical toxicity solely from a chemical's molecular structure. In contrast, our predictor is a unique specialized tool for the prediction of 5-HT_{2B} activity and therefore prioritizing compounds for functional testing against 5-HT_{2B} receptors to assess their valvulopathic potential. Therefore, this predictor can be used, along with other computational chemical health risk assessment tools, to evaluate compounds' safety at early stages of the drug development. It can be used as well to verify that all drugs available on the market are free from possibly fatal valvulopathic risk. This predictor will be made publicly available at the ChemBench server established in the Laboratory for Molecular Modeling (chembench.mml.unc.edu). We will also gladly apply this predictor to any compound library that may be of interest to any researcher.

Experimental Section

Radioligand Binding Assays

This screen was performed by the National Institute of Mental Health Psychoactive Drug Screening Program (PDSP). Radioligands were purchased by PDSP from Perkin-Elmer or GE Healthcare. Competition binding assays were performed using transfected or stably expressing cell membrane preparations as previously described (Shapiro *et al.* 2003;[90] Roth *et al.* 2002[91]) and are available online (http://pdsp.med.unc.edu). All experimental details are available online (http://pdsp.med.unc.edu/UNC-CH%20Protocol%20Book.pdf).

Chemistry

Chemical compounds predicted as hits from the virtual screening were obtained from commercial suppliers according to their availability. All compounds were ordered to have ≥ 95% purity. Additionally, all compounds were subjected to purity assessment using LC/MS by the Center for Integrative Chemical Biology and Drug Discovery at UNC-Chapel Hill. LC/MS spectra of all compounds were acquired from an Agilent 6110 Series system with UV detector set to 220 nm. Samples were injected (5 uL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 uM, C18 column at room temperature. A linear gradient from 10% to 100% B (MeOH + 0.1% Acetic Acid) in 5.0 min was followed by pumping 100% B for another 2 minutes with A being H_2O + 0.1% acetic acid. The flow rate was 1.0 mL/min.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

5-HT_{2B}	5-Hydroxy Tryptamine subtype 2B receptors
AD	Applicability Domain

CARs	Class Association Rules
CBA	Classification Based on Association
CCR	Correct Classification Rate
CCR_{train}	Correct Classification Rate for training set
CCR_{test}	Correct Classification Rate for test set
CCR_{evs}	Correct Classification Rate for external validation set
CCR_{ex}	Correct Classification Rate for external set
CCR_{rand}	Correct Classification Rate of the random models using the external validation set
CV	Cross Validation
DWD	Distance Weighted Discrimination
E	Enrichment
En	Normalized Enrichment
FN	False Negative
FP	False Positive
HTS	High Throughput Screen
***k*NN**	K Nearest Neighbor
LOO-CV	Leave-One-Out Cross Validation
MFD	Most Frequent Descriptors
MOE	Molecular Operating Environment
MZ	MolConnZ descriptors
PDSP	NIMH Psychoactive Drug Screening Program
QSAR	Quantitative Structure Activity Relationships
SA	Simulated Annealing
SE	Sensitivity
SG	Subgraph
SP	Specificity
TP	True Positive
TN	True Negative
VHD	Valvular Heart Disease
VS	Virtual Screening
WDI	World Drug Index

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Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:11934–11939. [PubMed: 12192085]

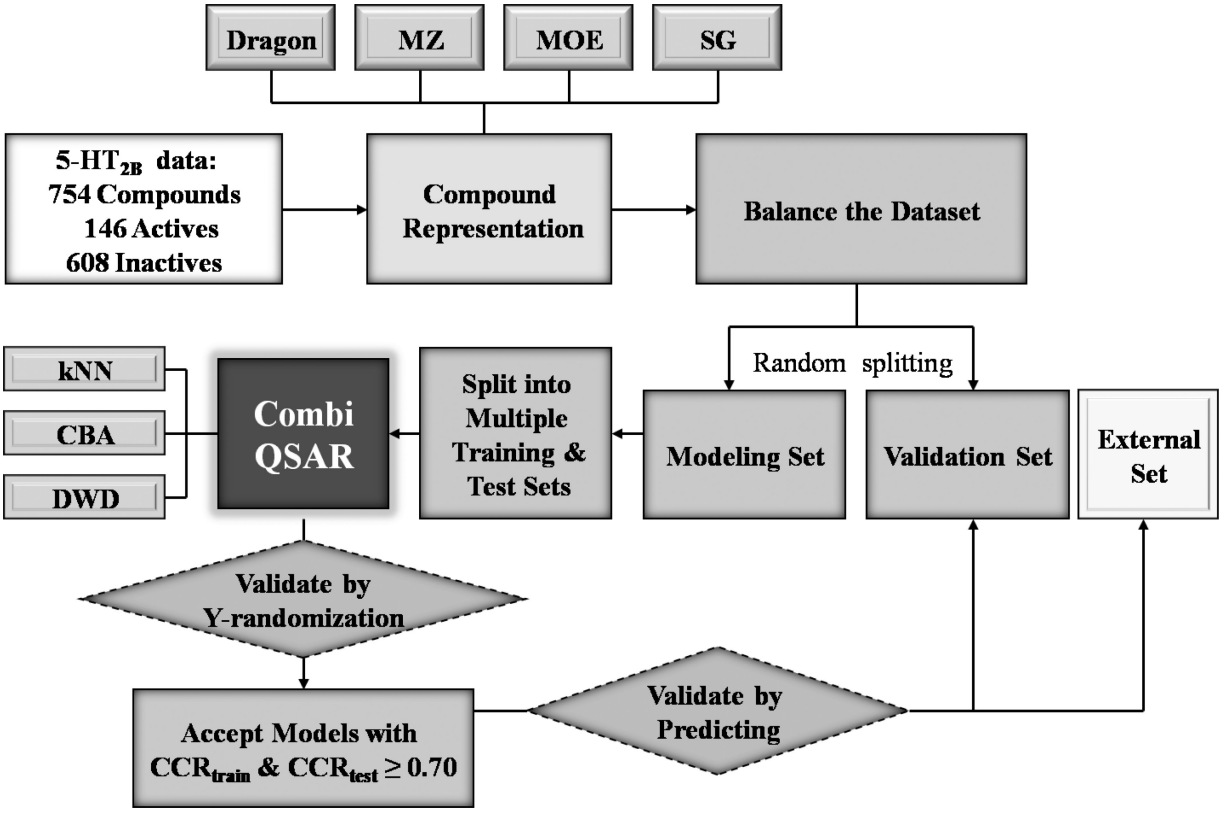


Figure 1.
The workflow for QSAR model building and validation as applied to the 5-HT_{2B} dataset (see text for abbreviations).

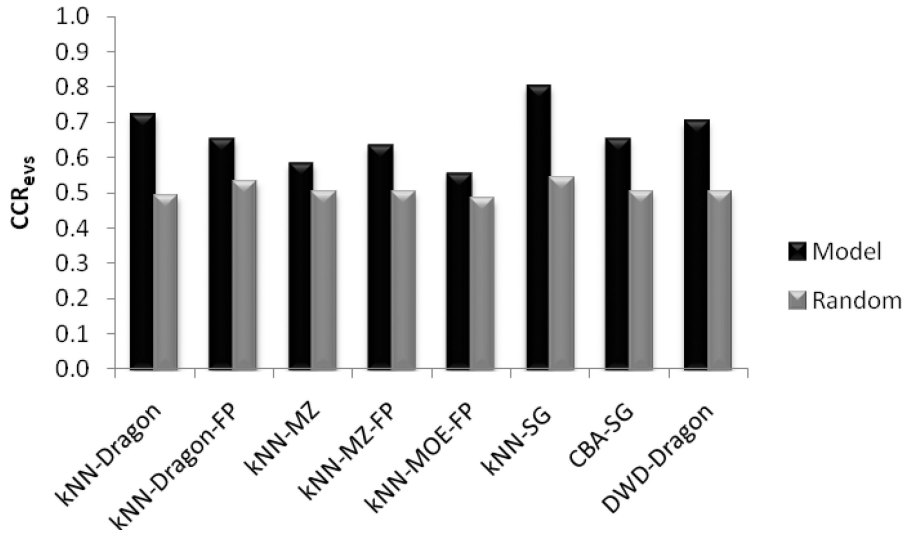


Figure 2.

Comparison of CCR values for the external validation set (CCR_{evs}) for different QSAR models developed to classify actives *vs.* inactives. CCR_{evs} values for models built with both real (blue) and randomized (red) activities of the training sets are shown (see text for abbreviations).

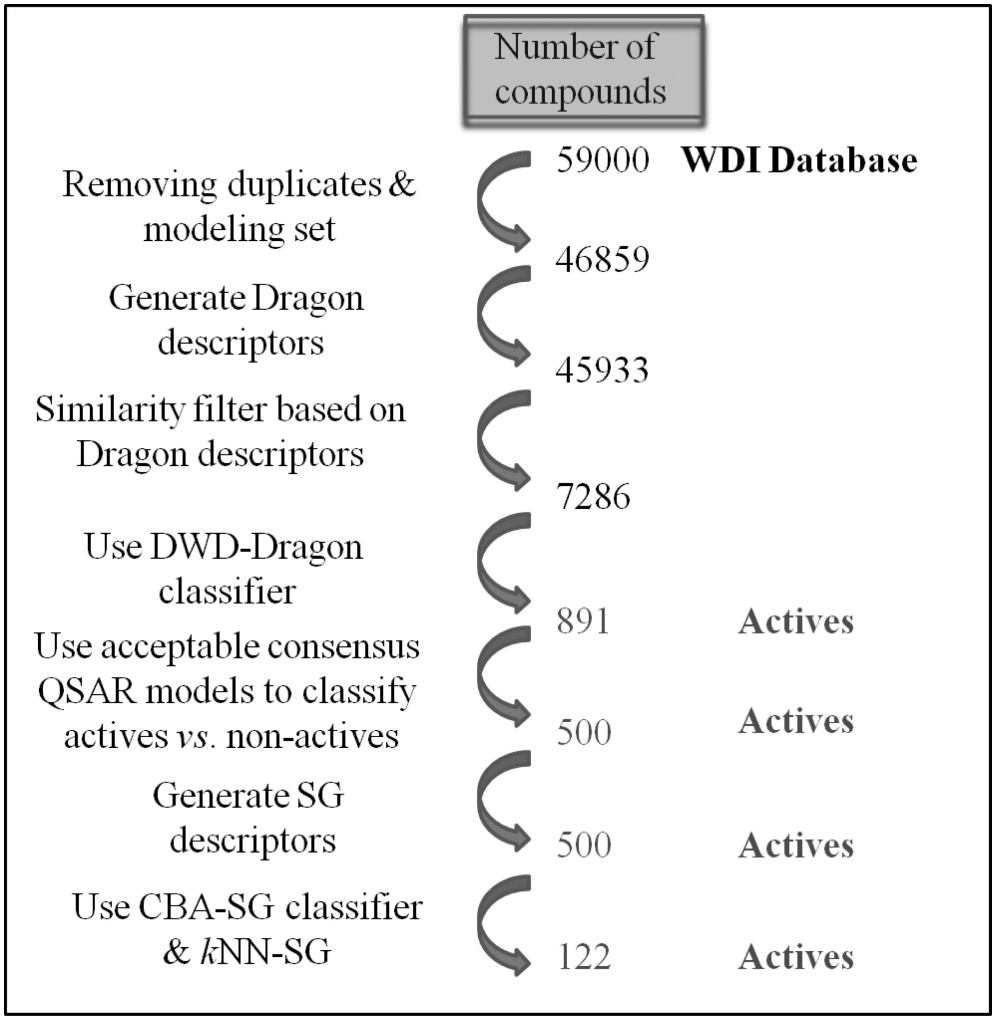


Figure 3.
Steps of the virtual screening of the WDI database to identify putative 5-HT_{2B} ligands (see text for the abbreviations).

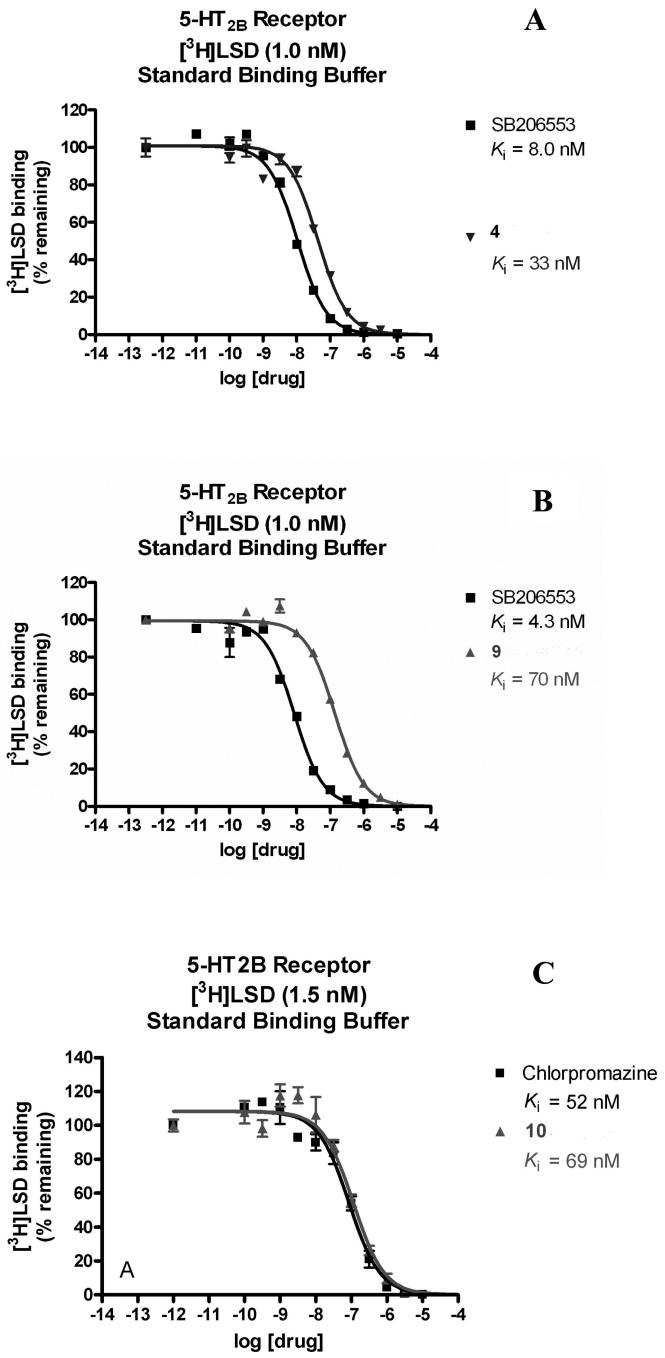
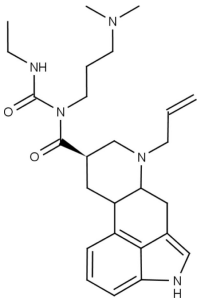
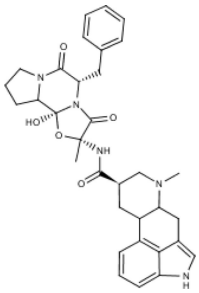
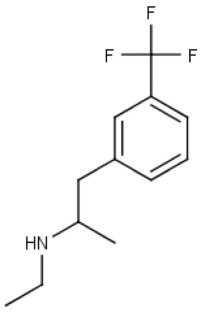
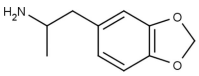
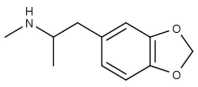


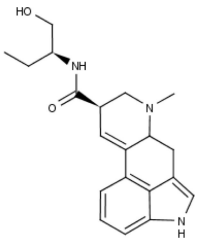
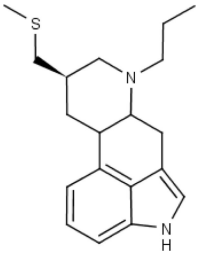
Figure 4.

Competition binding at 5-HT_{2B} receptors for (A) **4** (triangle) and SB206553 (square), (B) **9** (triangle) and SB206553 (square), and (C) **10** (triangle) and chlorpromazine (square), versus [3H]LSD.

Table 1

Chemical structures of marketed drugs known as 5-HT_{2B} receptor agonists and associated with VHD.

Compound	PubChem CID	5-HT_{2B} Agonist	VHD
 Carbergoline	54746	Yes	Yes
 Dihydroergotamine	10531	Yes	Yes
 Fenfluramine	3337	Yes	Yes
 MDA	1614	Yes	??[a]
 MDMA	1615	Yes	Yes

Compound	PubChem CID	5-HT$_{2B}$ Agonist	VHD
 Methylergonovine	8226	Yes	Yes
 Pergolide	47811	Yes	Yes

[a] Unknown.

Table 2

Performance of *k*NN classification methods to classify actives *vs.* inactives based on external validation set statistics.

Model	Num. Models[a]	Confusion Matrix						Statistics for the Models					
		N(1)[b]	N(0)[c]	TP	TN	FP	FN	SE	SP	*En*(1)	*En*(0)	CCR_{evs}[d]	CCR_{rand}[e]
A[f]	908	26	34	20	23	11	6	0.77	0.68	1.41	1.49	0.72	0.49
B[g]	235	38	36	22	20	16	16	0.58	0.56	1.13	1.14	0.57	0.50
C[h]	619	32	38	17	29	9	15	0.53	0.76	1.38	1.24	0.65	0.53
D[i]	387	30	40	16	29	11	14	0.04	0.73	0.26	1.90	0.63	0.50
E[j]	123	30	40	20	26	14	10	0.67	0.65	1.31	1.32	0.66	0.46
F[k]	93	30	40	23	33	7	7	0.77	0.83	1.63	1.56	0.80	0.54

[a] Num. Models, number of models with CCR_{train} and $CCR_{test} \geq 0.70$

[b] N(1), number of actives

[c] N(0), number of inactives

[d] CCR_{evs}, correct classification rate of the consensus models using the external validation set

[e] CCR_{rand}, correct classification rate of the random models using the external validation set

[f] A, *k*NN-Dragon

[g] B, *k*NN-MZ

[h] C, *k*NN-Dragon-FP

[i] D, *k*NN-MZ-FP

[j] E, *k*NN-MOE-FP

[k] F, *k*NN-SG.

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Table 3

Comparison between different *k*NN-Dragon QSAR models generated with or without variable selection.

Model	Num. Models[a]	Confusion Matrix						Statistics for the Models					
		N(1)[b]	N(0)[c]	TP	TN	FP	FN	SE	SP	*En*(1)	*En*(0)	CCR_{evs}[d]	Coverage[e]
A[f]	908	26	34	20	23	11	6	0.77	0.68	1.41	1.49	0.72	100%
B[g]	1	26	34	10	22	10	8	0.38	0.65	1.13	1.36	0.52	83%
C[h]	1	26	34	14	15	19	9	0.54	0.44	0.98	1.12	0.49	95%
D[i]	1	26	34	14	15	19	9	0.54	0.44	0.98	1.12	0.49	95%

[a] Num. models, number of models with CCR_{train} and $CCR_{test} \geq 0.70$

[b] N(1), number of actives

[c] N(0), number of inactives

[d] CCR_{evs}, correct classification rate of the consensus models using the external validation set

[e] Coverage: percentage of predicted compounds, and coverage = % of the external set compounds predicted by the models

[f] A, *k*NN-Dragon

[g] B, *k*NN-Dragon-NVS where *k*NN model was generated using all 387 Dragon descriptors with no variable selection and 1 nearest neighbor (NN)

[h] C, *k*NN-Dragon-MFD where the *k*NN model was generated with top 20 most frequent Dragon descriptors and 1NN

[i] D, *k*NN-Dragon-DWD where the *k*NN model was generated with top 20 highly weighted Dragon descriptors by DWD and 1 NN.

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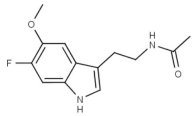
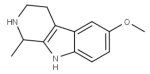
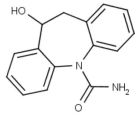
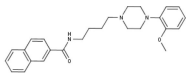
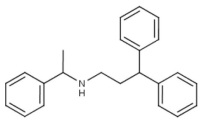
Table 4

Experimental validation results for the 10 computational hits predicted as 5-HT_{2B} ligands as a result of QSAR-based mining of the WDI chemical screening library.

Compound ID	Chemical Stucture/Name	PubChem CID	PDSP ID	Predicted 5-HT_{2B} Activity	Experimental *Ki* (nM)
1	 6-Fluoromelatonin[88]	43922	14809	Active	2,495
2	 Adrenoglomerulotropin[88]	71028	14807	Active	491
3	 CGP-13698[88]	114709	14806	Active	>10,000
4	 DO-897[88]	3038495	14814	Active	33.1
5	 Fendiline[88]	3336	14821	Active	3,217

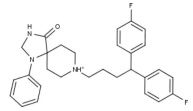
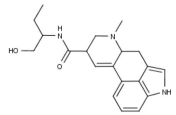
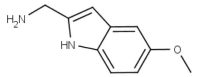
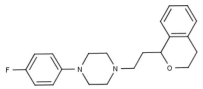
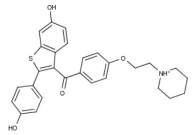
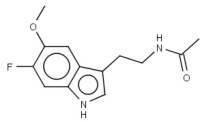
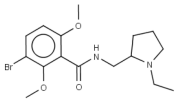
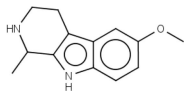
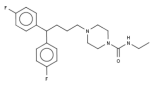
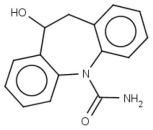
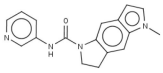
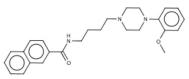
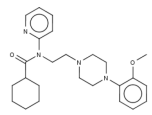
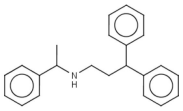
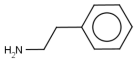
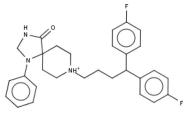
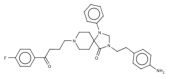
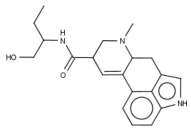
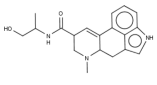
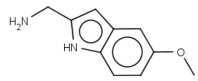
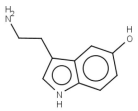
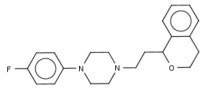
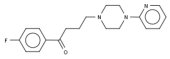
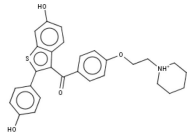
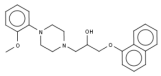
Compound ID	Chemical Stucture/Name	PubChem CID	PDSP ID	Predicted 5-HT$_{2B}$ Activity	Experimental *Ki* (nM)
6	 Fluspirilene[88]	1715104	14815	Active	151.4
7	 Methylergometrine[88]	4140	27769	Active	0.8
8	 PIM-35[88]	195658	14805	Active	1,617
9	 PNU-96415E[88]	9909648	13513	Active	69.6
10	 Raloxifene[88]	15940170	13505	Active	69

Table 5

Nearest neighbor compounds from the active compounds in the dataset and the 10 experimentally validated VS hits.

Virtual screening hits	Nearest neighbor from the modeling set compounds based on MACCS structural keys and Tanimoto distances
 1	 PubChem CID 54940 (66 % similarity)
 2	 PubChem CID 108029 (70 % similarity)
 3	 PubChem CID 5163 (49 % similarity)
 4	 PubChem CID 5684 (88 % similarity)
 5	 PubChem CID 1001 (71 % similarity)
 6	 PubChem CID 125085 (90 % similarity)
 7	 PubChem CID 3250 (98 % similarity)

Virtual screening hits	Nearest neighbor from the modeling set compounds based on MACCS structural keys and Tanimoto distances
 **8**	 PubChem CID 5202 (76 % similarity)
 **9**	 PubChem CID 15443 (79 % similarity)
 **10**	 PubChem CID 4418 (74 % similarity)