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Discovery of Natural Product-derived 5-HT_{1A} Receptor Binders by Cheminformatics Modeling of Known Binders, High Throughput Screening and Experimental Validation

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Abstract

The human 5-hydroxytryptamine receptor subtype 1A (5-HT_{1A}) is highly expressed in the *raphe* nuclei region and limbic structures; for that reason 5-HT_{1A} had served as a promising target for treating human mood disorders and neurodegenerative diseases. We have developed binary quantitative structure-activity relationship (QSAR) models for 5-HT_{1A} binding using data retrieved from the WOMBAT database and the *k*-Nearest Neighbor (*k*NN) machine learning method. A rigorous QSAR modeling and screening workflow had been followed, with extensive internal and external validation processes. The models' classification accuracies to discriminate 5-HT_{1A} binders from the non-binders were as high as 96% for the external validation. These models were employed further to mine two major natural products screening libraries, i.e. TimTec Natural Product Library (NPL) and Natural Derivatives Library (NDL). In the end five screening hits were tested by radioligand binding assays with a success rate of 40%, and two new compounds were confirmed to be binders at the μ M concentration against the human 5-HT_{1A} receptor. The combined application of rigorous QSAR modeling and model-based virtual screening presents a powerful means for profiling natural products compounds with important biomedical activities.

GRAPHICAL ABSTRACT

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CONFLICT OF INTEREST

The authors confirm that they do not have any conflicts of interest.

Keywords

5-HT1A receptor; high-throughput screening; natural products; QSAR; external validation; profiling activities

INTRODUCTION

The human 5-HT_{1A} receptor is a subtype of 5-HT receptor that binds the endogenous neurotransmitter serotonin (5-hydroxytryptamine, 5-HT)¹. It is the most widespread of all the 5-HT receptors, an important family of G protein-coupled receptors (GPCRs). In the central nervous system (CNS), 5-HT_{1A} receptor exists in the cerebral cortex, hippocampus, septum, amygdala and raphe nucleus in high densities, while it is also located at the basal ganglia and thalamus in low amounts². It had been among the most important molecular targets that were actively being pursued for drug discovery efforts in psychoactive treatment³. Because of its dense concentration on cortical and hippocampal pyramidal neurons, 5-HT_{1A} receptor had been actively studied in recent years for novel strategies to treat the cognitive deficits in schizophrenia⁴. In fact, atypical antipsychotic drugs modestly enhance cognition and several drugs in this class were found to show 5-HT_{1A} partial agonist activity (e.g. aripiprazole, clozapine, olanzapine, ziprasidone and quetiapine)^{5,6,7}. They can sometimes be used in low doses as augmentation to standard antidepressants, for example, the selective serotonin reuptake inhibitors (SSRIs)⁸. In addition, 5-HT_{1A} receptor agonists such as buspirone, tandospirone and flesinoxan showed efficacy in relieving anxiety and depression^{9,10}, and some have been approved for these indications in various parts of the world. Other drugs like gepirone, flesinoxan^{11,12}, flibanserin^{13,14} and PRX-00023^{15,16} were also investigated, though none of them had been fully developed and approved so far.

The 5-HT_{1A} receptor has recently received considerable attention for its potential treatments to neurodegenerative diseases. 5-HT_{1A} receptor activation had been shown to increase dopamine release in the medial prefrontal cortex, striatum and hippocampus, and may be useful for improving the symptoms of Parkinson's disease¹⁷. As mentioned above, some of the atypical antipsychotics are 5-HT_{1A} receptor partial agonists, and this property had been shown to enhance their clinical efficacy. They act by enhancing dopamine release which plays a major role in the antidepressant and anxiolytic effects seen upon postsynaptic activation of the 5-HT_{1A} receptor¹⁸. Moreover, 5-HT_{1A} receptor antagonists such as lecozotan have been shown to facilitate certain types of learning and memory in rodents by

stimulating the release of glutamate and acetylcholine in various areas of the brain¹⁹. As a result, they are now being developed as novel treatments for Alzheimer's disease. Although several drugs including the ones mentioned above are available on market and show the affinities to human 5-HT1A receptor, few were developed specifically for modulating this target. They were either insufficient in term of efficacy for some patients or exhibit various side effects for others. Taken together, there is still a critical need in developing novel 5-HT1A receptor modulators to benefit the aforementioned diseases.

High throughput screening (HTS) has become a popular and efficient approach for the discovery of lead compounds in recent years. Once a quantitative structure-activity relationship (QSAR) model was built, it can be employed to mine large libraries of small molecules with the goal of identifying drug-like, structurally novel hits with the desired biological efficacy²⁰. Recently, we have demonstrated that QSAR models can be used in HTS, also known as database mining, *i.e.* finding molecular structures that are similar in their activities to the probe molecules or even profiling the activities of compounds in a library^{21222324,25}. At the same time, the number of chemicals available for HTS has been growing significantly over the past decade. This constant increase in the size of chemicals libraries available highlights the importance of classifying chemical libraries by their characteristics, so that HTS can be performed accordingly in regards to different objectives.

MATERIALS AND METHODS

Datasets for QSAR Model Building

The data on 5-HT1A binders were retrieved from the WOMBAT database while we collected 5-HT1A non-binders from the Psychoactive Drug Screening Program (PDSP) at the National Institute of Mental Health (NIMH). During this study, we employed 10 μ M as the threshold value to specify binders *vs.* non-binders, and only retrieved ligands tested against cloned human species cell lines using the hot ligand of [³H]-8-OH-DPAT. By submitting such queries, we designated 69 unique compounds as 5-HT1A binders with binding affinities stronger than 10 μ M. We also retrieved 61 non-binders that were shown to have no binding affinities to 5-HT1A receptor at 10 μ M concentration.

Dataset Curation

For the purposes of reliable modeling, our datasets were curated following the protocols published earlier²⁶. In the beginning, we cleaned all compounds with the Wash Molecules module in Molecular Operating Environment (MOE²⁷, version 2009.10). This module processes 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 can be found in the MOE manual²⁷). Second, ChemAxon Standardizer²⁸ was used to harmonize the representation of aromatic rings. Finally, the structural duplicates were detected by the analysis of the normalized molecular topologies. The functional data for duplicated compounds were verified to be identical, so in each case only a single data entry was retained. The curated subset of the original 5-HT1A dataset used in this work included 130 unique organic compounds including 69 binders and 61 non-binders.

Natural Products Chemical Libraries

TimTec (<http://www.timtec.net/>) Natural Product Library (NPL) is a chemical library of 720 natural compounds composed of pure natural products as lead identifying materials. It includes primarily known natural compounds that are also available through a number of domestic and international commercial sources. The value of the library design is in the broad diversity of selected natural material available in a screen-ready format. TimTec does not hold any intellectual property rights for compounds in this collection.

TimTec Natural Derivatives Library (NDL) elaborates on structural variety of pure natural compounds and includes synthetic compounds as well as synthetically modified pure natural compounds: alkaloids, natural phenols, nucleoside analogs, carbohydrates, purines, pyrimidines, flavonoids, steroidal compounds and natural amino acids. It is a “natural” extension of the aforementioned NPL, in both design and structural diversity. It should be noted that there is no overlap between NPL and NDL compounds. All NDL compounds comply with screening purity standards and are available as a collection of either 3,040 individual compounds, or smaller subsets.

Selection of Training, Test, and External Validation Sets

As shown in Fig. 1, we followed the rigorous QSAR workflow for model building, validation and screening established earlier²⁹. For this classification QSAR modeling, we have employed five-fold external cross-validation (CV) protocol, i.e. the sample set of 166 compounds was divided randomly into five subsets, with one subset used for external testing and the other four for model training and internal testing. This procedure was repeated five times and a different one-fifth of the dataset was used for external testing each time. The remaining compounds were considered as modeling dataset; they were further partitioned into multiple pairs of chemically diverse and representative training and test sets of different sizes, using the sphere exclusion algorithm adapted to QSAR modeling efforts^{30,31}.

Generation of 2D Molecular Descriptors

The SMILES³² strings of each compound in the 5-HT1A dataset were converted to 2D chemical structures using the MOE package. The Dragon software³³ (version 5.5) was used to calculate a wide range of topological indices of molecular structure. These indices include but not limit to the following descriptor types: simple and valence path, cluster, path/cluster and chain molecular connectivity indices, kappa molecular shape indices, topological and electro-topological state indices, differential connectivity indices, graphs' radius and diameter, Wiener and Platt indices, Shannon and Bonchev-Trinajsti information indices, counts of different vertices, counts of paths and edges between different kinds of vertices³³.

Overall, Dragon generated over 2,000 different molecular descriptors. Most of these descriptors characterize chemical structure, but several depend upon the arbitrary numbering of atoms in a molecule and are introduced solely for bookkeeping purposes. In our study, about 880 chemically relevant descriptors were initially calculated and 672 descriptors were eventually employed for this 5-HT1A binder/non-binder dataset after deleting descriptors with zero value or zero variance. All Dragon descriptors were range-scaled prior to distance calculations since the absolute scales for Dragon descriptors can differ by orders of

magnitude. Accordingly, our conversion by range-scaling avoided giving descriptors with significantly higher ranges a disproportional weight upon distance calculations in multidimensional Dragon descriptor space.

k-Nearest Neighbor (kNN) Classification Algorithm

In pattern recognition, the *k*NN classification QSAR method³⁴³⁵ is a non-parametric method used for classification and regression. In *k*NN classification, the output is a class membership. An object is classified by a majority vote of its neighbors, with the object being assigned to the class most common among its *k* nearest neighbors (*k* is a positive integer, typically small). If *k* = 1, then the object is simply assigned to the class of that single nearest neighbor. In our cases, the similarity is calculated using only a subset of all descriptors, which is optimized by simulated annealing (SA) technique in order to reach the best Correct Classification Rate (CCR)³⁶:

$$CCR = \frac{1}{2} \left(\frac{N_1^{corr}}{N_1^{total}} + \frac{N_2^{corr}}{N_2^{total}} \right) \quad (1)$$

where N_1^{total} and N_2^{total} are the number of binders and non-binders in the dataset, N_1^{corr} and N_2^{corr} are the number of known binders predicted as binders (i.e. true positives) and the number of non-binders predicted as non-binders (i.e. true negatives). The statistical significance of the predictive models is defined by the leave-one-out cross-validation (LOO CV), i.e. CCR_{train} for the training sets and CCR_{test} for the test sets. Models of high CCR_{train} and CCR_{test} were used as a matter of external validation, i.e. to predict compounds included neither in the training nor in the test set. In theory, any chemicals represented by their corresponding molecular descriptors can be predicted to a class using the *k*NN classification approach. Formally, a QSAR model can predict the target property for any compound for which chemical descriptors can be calculated. However, if a compound is highly dissimilar from all compounds of the modeling set, reliable prediction of its activity is unlikely to be realized. A concept of similarity threshold (or applicability domain, AD) was developed and used to avoid such an unjustified extrapolation in activity prediction³⁷. In our studies, the AD is defined as the Euclidean distance threshold DT between a query compound and its closest *k*-nearest neighbors of the training set. It is calculated as follows:

$$D_T = \bar{y} + Z\sigma \quad (2)$$

Here, $\bar{y}$ is the average Euclidean distance between each compound and its *k* nearest neighbors in the training set which is also optimized in the course of QSAR modeling, σ is its standard deviation of these Euclidean distances, and *Z* is an arbitrary parameter called the Z_{cutoff} defined by a user. Typically, we set *Z* to 0.5, which places the boundary for deciding whether a compound is within or outside of the AD at one half of the standard deviation from $\bar{y}$. We also define the AD in the entire descriptor space. Thus, if the distance of the external compound from its nearest neighbor in the training set within either the entire descriptor space or the selected descriptor space exceeds these thresholds, the prediction is not made. It is important to note that raising the value of *Z* would increase the number of

molecules in the external set that are by definition inside the AD but could reduce the prediction accuracy because of the inclusion of dissimilar compounds.

Robustness of QSAR Models

The Y-randomization test has been widely used to ensure the robustness of QSAR models³⁸. To establish model robustness, Y-randomization (randomization of the response variable) test should be used. This test consists of repeating all the calculations with scrambled activities of the training set. Ideally, calculations should be repeated at least five (better, more) times. The goal of this procedure is to establish whether models built with real activities of the training set have good statistics not due to overfitting or chance correlation. If predictive power for the training (i.e. LOO CV CCR_{train}) or the test set (i.e. CCR_{test}) of all models built with randomized activities of the training set is significantly lower than that of models built with real activities of the training set, the latter ones are considered reliable. In the end, we applied the one-tail hypothesis to confirm the robustness of our QSAR models.

HTS using Consensus Models

As illustrated in the workflow of Fig. 1, QSAR models that passed both internal and external validation were employed for our HTS purpose³⁹. A global AD defined by the whole body of descriptor types was applied first in order to filter out compounds that are structurally distant from compounds in the modeling set. All 69 known 5-HT_{1A} binders extracted from WOMBAT were used as probes for the AD calculations. Subsequently, the consensus prediction of *k*NN ensemble models was conducted only to compounds falling into the global AD. The results were deemed to be acceptable only when the compound was found within the AD of more than 50% of all models employed in consensus prediction and the standard deviation of estimated means across all models was small.

All the modeling and virtual screening calculations were carried out at an Intel Xeon X5667 processor-based, 2-socket rack server Dell PowerEdge R410 and multiple Dell Precision M6500N Mobile TAA workstations (Intel i7-840QM Quad Core), at the Laboratory of Cheminformatics and Drug Design (LCDD) at Howard University, also the Molecular Modeling and Drug Discovery Core for DC D-CFAR. Part of the calculations were conducted at a 352-processor Beowulf Linux cluster of the ITS Research Computing Division of the University of North Carolina at Chapel Hill (UNC-CH). The cluster runs the Red Hat Enterprise Linux 4.0 (32-bit) and the nodes communicate via a Gigabit Ethernet network.

Radioligand Binding Assays

The experimental validation was performed by the NIMH 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 described⁴⁰⁴¹ and are available online at <http://pdsp.med.unc.edu>. All experimental details are available at PDSP's portal site, <http://pdsp.med.unc.edu/UNC-CH Protocol Book.pdf>.

RESULTS AND DISCUSSION

QSAR Classification Models

The *k*NN classification method yielded with great success a set of acceptable QSAR models with high accuracy characterized as CCR for both training and test sets. For each of the individual modeling set out of the five-fold splits, we found that there are over seven models of both CCR_{train} and CCR_{test} equal to or higher than 0.80. Notably, most models of CCR_{test} greater than 0.80 also showed their corresponding CCR_{train} greater than 0.80 for the current data sets, though the opposite was not always true. According to our prior workflow, only models with prediction accuracy of both CCR_{train} and CCR_{test} greater than 0.80 are deemed acceptable and selected for consensus prediction. In current cases, their CCR_{train} and CCR_{test} were actually 0.91 and 0.99 respectively, suggesting that those models can distinguish precisely the majority of true-binders from non-binders for both training and test sets. These kinds of remarkably high prediction accuracies as well as the large number of eligible models indicate that our *k*NN modeling method was capable of telling apart true-binders *versus* non-binders with great success using Dragon topological descriptors.

QSAR Model Validations

In recent years, we applied Y-randomization and external validation to be critical steps of our whole QSAR/VS workflow in addition to the internal validation of *k*NN models by test sets. We exploited only those models that had been validated by the prior two steps for external prediction and database mining³⁷. Furthermore, we had another dataset of 105 5-HT1A binders from PDSP used as the independent external validation, to increase the confidence of our QSAR models. In the Y-randomization test conducted, the binary annotations of 5-HT1A binders and non-binders in training sets were randomly shuffled, and *k*NN classification models were built with the same parameter settings. The test was performed once for each training/test set split and all runs of Y-randomization test showed that almost all models had both CCR_{train} and CCR_{test} less than 0.50. Moreover, the one-tail hypothesis testing was applied and confirmed that the difference of CCR_{train} before and after Y-randomization was significant.

In this study, we employed the five-fold CV approach for external prediction, i.e. the 26 compounds are randomly excluded from the original set for each fold and each compound is assigned to the external set for once. Consensus predictions were carried out using those predictive models with CCR_{train} and CCR_{test} greater than 0.80 under different Z value cutoffs ($Z_{\text{cutoff}} = 0.5 \sim 3.0$). Because of the AD inherent to individual *k*NN QSAR models, the consensus prediction sometimes cannot cover whole compounds in the single fold. Table 1 shows the consensus scores for each of the five-fold external sets. The consensus score (or *k*NN score), in term of the average value of class number (1 for binders while 2 for non-binders) in classification QSAR, was calculated by the fraction of models that predict a compound as non-binder over the total number of models used for prediction plus 1. Under $Z_{\text{cutoff}} = 0.5$, most external validation sets achieved a rather high prediction accuracy. For the fourth external set split, the validation attained 100% for binders and 92% for non-binders, leading to CCR_{evs} = 0.96. The only falsely predicted binder (average class number > 1.5) was within the AD of small portion of all models, i.e., the model coverage was fairly

low and the prediction value is not greater than 1.7. In general, the prediction with such a low coverage is considered to be of low confidence level. The large value of Z_{cutoff} significantly raised the model coverage for binder/non-binder predictions because of the expanded AD for individual models. However, such kind of AD for consensus models also decreases confidence level of external prediction. Generally speaking, in order to have reliable and accurate prediction, the QSAR model has to have broad model coverage and a small Z_{cutoff} value. In summary, only k NN models with $\text{CCR}_{\text{train}}$, CCR_{test} and CCR_{evs} equal to or greater than 0.80 were applied to consensus prediction and virtual screening. In addition, the eligible models chosen for the external prediction had relatively small Z_{cutoff} ($= 0.5$) and relatively broad coverage for compounds in external datasets ($\geq 50\%$).

Independent External Validation

We employed models built from 130 5-HT1A binder/non-binder dataset to verify 105 new 5-HT1A binders from PDSP. We should emphasize that these recent binders are unique in structure from existing PDSP binders. Among the 105 binders, 70 compounds were within the AD of consensus k NN models using $Z_{\text{cutoff}} = 0.5$; 62 were accurately annotated by consensus prediction ($\text{CCR}_{\text{evs}} = 0.89$, cf. Table 1). Thus, the majority of ligands were predicted correctly by our consensus models. Since the falsely predicted 5-HT1A binders by k NN had the prediction values less than 1.7, and were within the AD of limited number of models, the k NN prediction is considered as of low confidence. The success of this independent external validation suggested that our QSAR models were predictive and robust enough to be applied to further virtual screening.

Here we should emphasize that our model validation is a critically inherent feature of our QSAR modeling workflow. The issue of model validation had been given a lot of attention by the cheminformatic research community in recent years⁴². Previously, most QSAR practitioners presumed that internally validated models built from available training set data should be externally predictive. It has been demonstrated, however, that conventional validation techniques including LOO CV or even leave-many-out (LMO) CV applied to the training set, internal validation as applied to the test set, are insufficient to ensure the external predictive power of QSAR models^{37,43}. Thus, we utilized five-fold external CV protocol in this study as well as the Y-randomization test to ensure the robustness and predictive power of k NN models. Needless to say, the use of externally validated models and AD is particularly critical when the models are employed in the practice of virtual screening.

Model-based HTS

Instead of applying only one and the best model to HTS, we followed the consensus prediction approach in our workflow by taking the average of all predictions from qualified models, i.e. seven models of both Internal and External $\text{CCR}_{\text{train}}$ and CCR_{test} equal to or greater than 0.80. We only kept the set of models from one particular split of the five-fold CV, the forth split in this case, which yields the highest CCR_{evs} . To narrow the hit list and obtain high confidence level for each prediction, we took both the consensus score and model coverage into consideration. In particular, only the screening hits of a consensus score between 1.0 and 1.1 and the model coverage over 50% were chosen. While these hits are

predicted to be 5-HT1A binders with high confidence by our consensus *k*NN models, it would be interesting to test them experimentally in hope of revealing new scaffolds of 5-HT1A binders. For these screening hits chosen by *k*NN models, five chemicals from NDL library were further selected for experimental validation based on the following criteria: 1) high confidence of consensus prediction by our models; 2) low structural similarity between hits and known 5-HT1A binders; 3) convenient commercial availability.

Experimental Validation

Using the competition binding assays with the radioligand of [³H]-8-OH-DPAT, five hit compounds were tested *in vitro* for experimental validation. The selection was based on high predicted activity, availability and structural uniqueness. Two tested compounds, CMPD#19546 and CMPD#19548, showed percent of inhibition of 84.3% and 70.9%, respectively (cf. Table 2). The further secondary experiments confirmed that their IC₅₀ are 2.86 μM for CMPD#19546 and 2.99 μM for CMPD#19548, as can be seen from the Fig. (2). We also employed the positive control of WAY100635, and its measured K_i value of 0.75 nM was consistent with the literature data. The comparison between predicted and experimental data is shown in Table 2. Apparently, our *k*NN prediction of CMPD#19546 is 1.04 ± 0.20 by an ensemble of 201 models (1: Active/2: Inactive), while for CMPD#19548 the prediction is 1.15 ± 0.36 out of an ensemble of 213 models. After searching chemical structure records in Chemical Abstracts Service (CAS) and PubChem databases, we did not find any prior reports of these two natural product-derived compounds' action on the human 5HT-1A receptor. It is once again proved that by taking advantage of various predictive tools, such as QSAR modeling, more novel compounds can be revealed with unreported biological activities.

CONCLUSIONS

The work reported above shows that our binary *k*NN classification models built with Dragon topological descriptors can characterize true 5-HT1A binders from non-binders with high accuracy. We applied our prior QSAR modeling workflow to these well-curated data sets and the models were validated in a rigorous manner, internally using multiple divisions of training/test set as well as Y-randomization, and externally using five-fold external CV sets. We have demonstrated that this strategy afforded a set of eligible QSAR models with supreme internal and external predictive power. Those qualified predictors (models) were further validated independently on a new set of PDSP binders, as part of our QSAR modeling workflow. It should be noted that our validation outcomes were highly consistent with the experimental activities of these compounds as 5-HT1A binders. In addition, we exploited these models in a highly conservative manner, i.e. in a consensus fashion and the strictest AD criteria, to screening two major screening libraries of natural products, i.e. NPL and NDL. Five of the screening hits were tested experimentally and two showed strong inhibition activities (>50%) at the single concentration of 10.0 μM. Further secondary assays confirmed that their binding constants of K_i are 2.86 μM and 2.99 μM, respectively. In summary, our current outcome indicates that when a sufficient amount of information on true binders versus non-binder is available, the QSAR modeling techniques can be favorably

applied not only to discriminating binders versus non-binders but most importantly, to profiling natural products-derived chemicals with unreported biomedical activities.

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List of Abbreviations

5-HT_{1A}	5-Hydroxy Tryptamine receptor subtype 1A
AD	Applicability Domain
CCR	Correct Classification Rate
CCR_{train}	Correct Classification Rate for training set
CCR_{test}	Correct Classification Rate for test set
CCR_{revs}	Correct Classification Rate for external validation set
CNS	central nervous system
CV	Cross-Validation
FN	False Negative
FP	False Positive
GPCRs	G-protein Coupled Receptors
HTS	High Throughput Screening
kNN	k Nearest Neighbor
LOO	Leave-One-Out
LMO	Leave-Many-Out
LOO CV	Leave-One-Out Cross-Validation
MOE	Molecular Operating Environment
NIMH	National Institute of Mental Health
PDSP	Psychoactive Drug Screening Program
QSAR	Quantitative Structure-Activity Relationship
SA	Simulated Annealing
SAR	Structure-Activity Relationship
SE	Sensitivity

SP	Specificity
SSRIs	Selective Serotonin Reuptake Inhibitors
TN	True Negative
TP	True Positive
WDI	World Drug Index
WOMBAT	World of Molecular Bioactivity

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Fig. (1). The workflow of cheminformatics models building, validation and virtual screening of natural product-derived hits as applied to the 5-HT1A dataset.

Fig. (2).

The full dose response curve for NDH hit compounds ST076806 (CMPD#19546; arrow-down triangles in Upper Panel; $K_i = 2,860$ nM), ST074727 (CMPD#19548; arrow-up triangles in Lower Panel; $K_i = 2,999$ nM), and the positive control, WAY100635 (solid squares in both Panels, $K_i = 0.75$ nM) measured by human 5-HT_{1A} receptor radioligand binding assay. The radioligand is $[^3\text{H}]\text{-8-OH-DPAT}$ at the concentration of 0.5 nM with the standard binding buffer.

Table 1

The validation results for the five-fold external sets as well as the additional independent external set from PDSP using the *k*-Nearest Neighbor method.

<i>a</i> External Sets	Prediction CCR	Confusion Matrix							Statistics		
		N(1) ^a	N(2) ^a	TP	TN	FP	FN	SE	SP	EN(1)	EN(2)
1	0.93	15	12	14	11	1	0	0.93	0.92	1.93	1.86
2	0.92	13	12	12	11	2	1	0.92	0.91	1.69	1.85
3	0.91	15	11	15	9	2	0	1.00	0.82	1.69	2.00
4	0.96	13	13	13	12	1	0	1.00	0.92	1.86	2.00
5	0.96	13	13	13	12	1	0	1.00	0.92	1.86	2.00
PDSP	0.89	70 ^b	0	62	N/A	N/A	8	0.89	N/A	N/A	N/A

^aN(1) = number of binders, N(2) = number of non-binders, TP = true positive (binders predicted as binders), FP = false positives (non-binders predicted as binders), FN = false negatives (binders predicted as non-binders), TN = true negative (non-binders predicted as non-binders), SE = sensitivity = TP/N(1), SP = specificity = TN/N(2), EN - the normalized enrichment, EN(1) = (2TP * N(2))/(TP * N(2) + FP * N(1)), EN(2) = (2TN * N(1))/(TN * N(1) + FN * N(2)), and CCR = correct classification rate.

^bSome N(1) binders from PDSP were out of the AD of the consensus models (Z_{cutoff} = 0.5), thus having no prediction. Only data for compounds found within the AD were used for statistical summaries.

Table 2

The prediction scores by kNN consensus models and experimental data for five hits identified by virtual screening as putative 5-HT1A binders.

Structure	No.	CMPD	Source library	Library ID	kNN score	SD	Percent of inhibition	Exp. IC ₅₀ (μM)
1	19546	NDL	ST076806	1.04	0.20	84.3	2.86
2	19547	NDL	ST026798	1.12	0.32	-6.7	ND
3	19548	NDL	ST074727	1.15	0.36	70.9	2.99
4	19549	NDL	ST049891	1.16	0.36	30.4	ND
5	19550	NDL	ST016609	1.16	0.36	32.1	ND

^aThe full IC₅₀ curve was generated in further experiment and the Ki value was determined.