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Developing Enhanced Blood–Brain Barrier Permeability Models: Integrating External Bio-Assay Data in QSAR Modeling

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Abstract

Purpose—Experimental Blood–Brain Barrier (BBB) permeability models for drug molecules are expensive and time-consuming. As alternative methods, several traditional Quantitative Structure–Activity Relationship (QSAR) models have been developed previously. In this study, we aimed to improve the predictivity of traditional QSAR BBB permeability models by employing relevant public bio-assay data in the modeling process.

Methods—We compiled a BBB permeability database consisting of 439 unique compounds from various resources. The database was split into a modeling set of 341 compounds and a validation set of 98 compounds. Consensus QSAR modeling workflow was employed on the modeling set to develop various QSAR models. A five-fold cross-validation approach was used to validate the developed models, and the resulting models were used to predict the external validation set compounds. Furthermore, we used previously published membrane transporter models to generate relevant transporter profiles for target compounds. The transporter profiles were used as additional biological descriptors to develop hybrid QSAR BBB models.

Results—The consensus QSAR models have $R^2=0.638$ for fivefold cross-validation and $R^2=0.504$ for external validation. The consensus model developed by pooling chemical and transporter descriptors showed better predictivity ($R^2=0.646$ for five-fold cross-validation and $R^2=0.526$ for external validation). Moreover, several external bio-assays that correlate with BBB permeability were identified using our automatic profiling tool.

Conclusions—The BBB permeability models developed in this study can be useful for early evaluation of new compounds (*e.g.*, new drug candidates). The combination of chemical and biological descriptors shows a promising direction to improve the current traditional QSAR models.

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Keywords

biological descriptors; blood–brain barrier; hybrid model; permeability

INTRODUCTION

The blood–brain barrier (BBB) separates the central nervous system (CNS) from the circulatory system and selectively limits many substances from entering the brain. The BBB is a sophisticated barrier system. Besides the tight junction and cell membranes that limit passive diffusion of molecular substances, the BBB is also composed of transporters that selectively regulate permeation of exogenous molecules (1).

The study of BBB permeability is crucial for drug development. While BBB permeability is required for CNS drugs to work (2), unexpected passage of a drug through BBB may cause severe side effects (3). Traditional experimental approaches to evaluate drug BBB permeability, such as animal testing, are expensive and time consuming. Therefore, alternative methods with significantly lower cost, such as *in vitro* or computational models, are desirable for drug research and development. Various computational models, especially those using Quantitative Structure–Activity Relationship (QSAR) approaches, have been developed in the past decades. Table SI shows QSAR models on the BBB permeability published within the last 5 years. However, the QSAR hypothesis that “chemically similar compounds tend to have similar activities” has its limitation when the modeling set is neither large and nor diverse (4). In small datasets, the existence of structurally similar compounds with vastly different activities, also called “activity cliffs,” greatly affects the predictivity of QSAR models (5).

With the development of high-throughput screening (HTS) techniques in the past decades, massive amounts of bio-assay data have become publically available. PubChem, the largest public data sharing portal, contains over 700,000 bio-assays with around 50 million compounds tested (6). A substantial number of PubChem bio-assays showed relevance to BBB permeability. For example, brain adenylate cyclase assays (PubChem AID 34292 and 34293) indicate binding affinity of this membrane-associated enzyme, which catalyzes the formation of the secondary messenger cyclic adenosine monophosphate (cAMP) and regulates the permeability in the brain capillaries (7). While the current “Big Data” pool is large, complex, and informative, there still exists a major challenge in how to apply these available comprehensive data on systemic biological models (*e.g.*, BBB permeability models) and benefit from it.

In this study, we address the above challenges by improving the predictivity of conventional QSAR models on BBB permeability using publicly available bio-assay data. To this end, we compiled a large quantitative BBB permeability database of 439 unique compounds (Supplementary files Table SII), which is larger than the training sets used in most of the previous modeling studies (Supplementary Table SI). After applying various modeling approaches (*i.e.*, *k*-nearest neighbor, random forest and support vector machine), the external predictivity of the resulting combinatorial QSAR model is comparable to previous developed models. Then, by applying the transporter assay data generated by our in-house

models (8) as biological descriptors, the predictivity of the resulting hybrid model was superior to the original QSAR models based only on chemical descriptors. Furthermore, we used our in-house automatic profiling tool (9) to generate a PubChem bio-assay profile for each compound in the dataset. The resulting profile contains 155 assays relevant to the BBB permeability. Although not suitable as additional descriptors due to missing data, some assays were able to provide possible explanations for some of the models' prediction outliers (compounds with large prediction errors).

METHODS

Dataset

A dataset of 484 compounds with experimental BBB permeability results was compiled from various public sources (10–13). The experimental values, which were represented as logBB (logarithm of brain-plasma concentration ratio at steady-state), range from –2.15 to 1.64 for these compounds. The chemical structure curation was performed using two chemical structure standardizer tools (Standardizer 6.3.0 from ChemAxon and CASE Ultra Datakurator 1.5.0.0 from Multicase Inc.) to remove duplicates, inorganics and mixtures. Since our descriptor generator cannot distinguish stereoisomers and salts, they will be considered to have the same chemical structures as their parent compounds. For this reason, duplicate compounds with different logBB values were carefully examined. In these cases, the 2-D structure of each stereoisomer was used and the larger component of the salt was neutralized and kept. This effort resulted in a curated logBB dataset consisting of 439 unique compounds. The source containing the largest number of compounds (total 362 compounds reported, 341 unique compounds after the curation) (10), was used as the modeling set in our study. The remaining 98 compounds were used as the external validation set. The distribution of the dataset by logBB ranges is shown in Fig. 1. Furthermore, after the QSAR models were developed, the compounds in this dataset were further classified as BBB permeable (logBB>0) or non-permeable (logBB ≤ 0). This arbitrary threshold used for classification was reported in several previous studies (14,15).

Overview of the Workflow in this Study

Figure 2 summarized the workflow designed for this study. After data curation, the QSAR approaches were applied to develop several QSAR logBB models. This procedure, framed red, represented the traditional QSAR modeling for the BBB permeability using rigorous external validation. Our in-house automatic profiling tool was used to extract all relevant biological response data for the compounds in the logBB dataset (framed by orange in Fig. 2). Then the chemical descriptors obtained from the chemical structures and the biological descriptors generated by the QSAR models of nine transporters were combined to develop an enhanced hybrid logBB model (framed by blue in Fig. 2).

Chemical Descriptors

The 2D Molecular Operating Environment (MOE) descriptors include physical properties, atom and bond counts, connectivity and shape indices, adjacency and distance matrix descriptors, subdivided surface areas, pharmacophore feature descriptors and partial charge descriptors, etc. A total of 192 2D descriptors were generated for each compound in the

dataset using MOE version 2013.08. After the descriptors were range-scaled to [0, 1], redundant descriptors were removed by deleting those with low variance (standard deviation <0.01) and/or randomly keeping one of any pairs of descriptors that have high correlation ($R^2 > 0.95$). The remaining 125 descriptors were used in the modeling process.

Modeling and Approaches

The QSAR models were developed using three different machine learning algorithms: Random Forest (RF), Support Vector Machine (SVM) and *k*-Nearest Neighbor (*k*NN). RF predictor consists of many decision trees and produces a prediction that combines the outputs from individual trees (16). SVM regression attempts to find the most narrow band in the descriptor-activity space containing most of the data points (17). We used standard implementation of RF and SVM algorithms as realized in R@0.2.15.1 using the package “e1071” (18). The settings of all statistical parameters to run these two algorithms were kept as default. The *k*NN (19) method uses weighted average of nearest neighbors as its prediction and employs variable selection procedure to define neighbors. It was developed using our in-house program implementation (20) (also available at chembench.mml.unc.edu). An extra consensus QSAR model was then generated by averaging predictions of the three individual models. The development and application of consensus QSAR models have been reported in our previous publications (21–23).

All models were validated using a five-fold cross-validation. Briefly, the modeling set was randomly divided into five equivalent subsets. One subset was used as the test set (20% of the modeling set compounds) and the remaining four subsets (80% of the modeling set compounds) were used as the training set. The training set was used to develop the QSAR models and the resulting models were validated by predicting the excluded test set. The procedure was repeated five times so that each modeling set compound was used in the test set once. Additional details regarding the QSAR modeling and validation procedure can be found elsewhere (24,25).

Integration of Biological Descriptors

We recently reported a QSAR modeling study for predicting chemical interactions of different Human Intestinal Transporters (HITs) (8). Some HITs presented on the BBB affect the permeability of compounds, *e.g.*, Apical Sodium-Dependent Bile Acid Transporter (ASBT) (26,27), Bile Salt Export Pump (BSEP) (28,29), Monocarboxylic Acid Transporters (MCT) (30), Multidrug Resistance Protein 1 (MDR1) (31), Multidrug Resistance-Associated Proteins (MRP1,3,4,5) (32), and Organic Anion Transporting Polypeptides (OATPs) (33). In this study, the predicted values were obtained from previously developed transporter models (8) available on chembench.mml.unc.edu, model ID: ASBT (112a, 112q, 112r), BSEP (242x, 242z), MCT1 (311q, 311x), MDR1 (313a, 313d, 313s, 313z), MRP1 (321x, 321z), MRP3 (333a, 333q, 333s, 333w), MRP4 (342x, 342z), MRP5 (344a, 344q), OATP 2B1 (413x, 413z). There are multiple QSAR models available for each transporter, thus the average predictions from individual models of each transporter were calculated and used in constructing the transporter profile. Finally, nine transporter activities were obtained for all 439 compounds in our database. None of the nine transporter activities correlated with each other for our data set and neither of them correlated with any of the 125 chemical descriptors

(standard deviation 0.01, R^2 0.95) that were used in the modeling process. Thus, the predicted activities of nine transporters were directly combined with the chemical descriptors to get the hybrid descriptors set. Then, the hybrid models were built based on the hybrid descriptor set using the same modeling approaches.

Additional bio-assay data was obtained from PubChem using our in-house automatic profiling tool (9). This tool aims to automatically extract experimental activities of PubChem assays for target compounds. The bioassays and their response data were kept when a bioassay has at least four active responses in our 439 compounds. The output file is a two-dimension matrix similar to the descriptor set used in the modeling process. The gathered bioassay data were then used for correlation analysis of BBB permeability. To simplify the identification and analysis of the bio-assays, the logBB values were categorized (logBB>0 as permeable with activity as 1, logBB ≤ 0 as non-permeable with activity as -1; see Fig. 1). The PubChem assays, with at least four compounds reported as permeable in our BBB database were kept for further analysis. Using this criterion, 310 PubChem assays and their response data were collected for the 275 compounds in our BBB database. Correlation of each bioassay to BBB permeability was calculated as the predictivity (number of true predictions over total number of known predictions) of this assay results to BBB permeability classifications. To further evaluate the correlation between bio-assay data and BBB permeability, a P_{sum} parameter was created as following:

$$P_{\text{sum}} = \text{Sum}(\text{Responses}) / N$$

In which, Sum(Responses) is the sum of the classified assay activity (1 for actives and -1 for inactives) for all compounds tested in this bio-assay and N is the number of these compounds. Thus, $P_{\text{sum}} > 0$ indicates that active response dominants while $P_{\text{sum}} < 0$ indicates negative response dominants.

RESULTS

Overview of the BBB Permeability Database

We analyzed the chemical space of the logBB dataset by performing a Principle Component Analysis (PCA) with the 192 MOE 2D descriptors used in this study. The top three most important components were used to generate a three-dimensional distribution plot for all 439 compounds (Fig. 3). Since these three components explained 59% of the total descriptor variance in this dataset, Fig. 3 can be viewed as the representation of chemical space covered by all compounds. There are several structural outliers, mostly non-permeable compounds. For example, Digoxin (PubChem CID 30322), which is widely used in heart failure treatment, was proven to be actively transported out of the BBB by MDR1 (34). Excluding structural outliers from the modeling set may improve robustness of the QSAR models (35), while outliers in the external set should be detected by the model's applicability domain (21,22). Since removing these structural outliers (*e.g.* Digoxin) did not show better modeling results (data not shown), and their logBB predictions might be improved after including biological descriptors, they were kept in this study. Using the same

three principle components, the Supplementary Figure S1 showed the chemical space distribution of both modeling and external validation sets.

Consensus QSAR Results

We developed three individual models and one consensus logBB model using the same modeling set. The performances of the models are represented by the five-fold cross-validation results and by predicting the external validation compounds (98 compounds not used in model development). The performances for all models are shown in Fig. 4. Among the individual models, the *k*NN model has a superior result for the five-fold external cross-validation ($R^2=0.690$ and $MAE=0.302$). However, the RF model has the best performance when predicting external compounds ($R^2=0.524$ and $MAE=0.399$). The conflicts between the results obtained from cross-validation and external prediction were reported in many previous QSAR studies (21,36). Meanwhile, using AD did not show improvement of results for five-fold cross-validation or external set predictions. We therefore retained all predictions (100% coverage). This condition makes it difficult to select the “top model” from various individual models for the purpose of external prediction. The consensus model (represented as CSS in Fig. 4), however, yielded better performance ($R^2=0.638$ $MAE=0.315$ in five-fold cross-validation, and $R^2=0.504$ $MAE=0.430$ in external validation) when compared to SVM and RF models in cross-validation, and SVM and *k*NN models in external prediction. Since it considers the output of all of the individual models without making model selection, yields better predictions for both cross-validation and external validation results than most individual models, the consensus model is more stable and reliable when predicting new compounds.

Bio-assay Data Improve Model Predictive Power

Our previous studies showed that using hybrid descriptors, which are the combinations of chemical and biological descriptors, showed superior results compared to traditional QSAR models only based on chemical descriptors (23,37,38). The predictivity of hybrid modes is higher than the traditional QSAR models and the analysis of chemical-biological descriptor patterns in resulting models can reveal the relevant chemical biological mechanisms of target activities. In this project, we assumed BBB permeability of a drug strongly depends on its biological interactions with active transporters on the BBB. Based on this hypothesis, we integrated the in-house transporter model predictions into our QSAR modeling process as extra biological descriptors. By combining the original chemical descriptors with transporter activities (as biological descriptors) into a hybrid (shown in Fig. 2), the predictivity of models in both the cross-validation and external prediction was improved. For the five-fold cross-validation, the results were improved for all three models. For example, in the SVM model, the R^2 value increased from 0.477 to 0.529, and the MAE decreased from 0.375 to 0.359 after including the transporter descriptors. Improvements were also observed in the external validation, with the exception of SVM. The *k*NN model, for instance, had the R^2 value improved from 0.464 to 0.520, and the MAE decreased from 0.440 to 0.422 for the prediction of validation set after including the transporter descriptors. The consensus model, regardless of modeling tools, also yielded the same trend of improvement in both cross-validation and external prediction (Fig. 4). The non-parametric paired permutation test ($N=10,000$ on MAE and R^2 metrics) using the in-house Matlab

script, which compares various performance metrics for two sets of matching predictions, showed that the improvement was significant for SVM ($p<0.001$) in five-fold cross-validation, RF and k NN and Consensus model ($p<0.05$) in external validation by paired permutation test comparison of MAE and R^2 , ($N=10,000$).

Driven by the benefit of including extra biological descriptors, we profiled 310 PubChem assays for the current BBB database. Figure 5 shows the correlation between these assays and BBB permeability. Assays were sorted by their correlation to the categorized logBB values for compounds in our BBB dataset. There are 144 assays (highlighted in orange dots on the bottom) with positive correlation to BBB permeability and 11 assays (highlighted in green dots on the top) with negative correlation to BBB permeability. In Fig. 5, according to the BBB permeability, the BBB database was divided into eight subsets, each containing 32–37 compounds. The difference of the P_{sum} value for each subset indicates the correlation of the relevant PubChem assay responses to the BBB permeability. At this time, this extra information cannot be applied to our modeling procedure due to missing data, as only 275 of 439 compounds in the data set were found to have at least four experimental data points from these assays as well as the incomplete profiles within the 275 compounds. Future QSAR modeling studies on these assays could supply the missing data and allow for this approach to be fully implemented.

DISCUSSION

Since the BBB is a complex biological system composed of diverse receptors, enzymes, and transporters, the traditional QSAR studies meet the bottleneck of predictivity. Models built on chemical descriptors (*e.g.*, MOE descriptors) obtained from a limited number of compounds are sometimes unable to distinguish two structurally similar compounds with different bio-activities (*i.e.*, logBB values). This “activity cliff” issue limits the application of computational predictive models that are based only on chemical descriptors (4).

The bio-assay responses of target compounds provide extra information that can be used to improve traditional QSAR models (37,38). Membrane transporters expressed in brain microvessels regulate the extent of flux and rate of exchange of substances between the circulatory system and CNS (39). Thus, a compound’s binding affinity to transporters will affect BBB permeability. As expected, our modeling results showed predictivity improvement by a simple combination of chemical descriptors and transporter descriptors (Fig. 4). This indicates that the improvements of prediction were due to information provided by the transporter data.

In order to interpret the mechanisms by which transporter interaction affects BBB permeability, we listed seven compounds that have better consensus predictions from the hybrid model compared to the conventional QSAR model, with their nearest neighbor compounds using chemical and transporter descriptors (Table I). The predicted activities for the nine transporters can be viewed as the transporter interaction profile for each compound (the range-scaled transporter interaction profiles are listed in the last column of Table I, blue: query compound, red: chemically nearest neighbor, green: combined nearest neighbor). The BBB permeability results, as well as transporter interaction profiles, indicate

that chemically similar compounds do not always have similar biological responses. For example, in group A composed of beta adrenergic receptor antagonists/agonists (Table I), Toliprolol (CID 18047, red line in transporter profile) is the most structurally similar compound to Atenolol (CID 2249, blue line in transporter profile), which is a drug used to treat hypertension, yet the BBB permeability and transporter interactions are quite different, especially for MRP4 and MRP5. However, after including the transporter descriptors, the new nearest neighbor Salbutamol (CID 2083), a drug used for the relief of bronchospasms, has the same BBB permeability and similar transporter interactions with Toliprolol. Similar conditions were also observed in group B and C (Table I). This can potentially be a solution to the “activity cliff” issue in QSAR studies (23,37,38). The differences in transporter interaction activities are able to differentiate the two structurally similar compounds in chemical space but with different bio-activities, thus correct the prediction for the query compound. Therefore, including meaningful biological descriptors (*e.g.*, transporter descriptors in this study) can improve the resulting models.

Through the analysis of the transporter interaction profiles, we are able to interpret the biological mechanisms of BBB permeability for specific compounds. For example, in group B of Table I, the query compound Doxorubicin (CID 31703, blue line in transporter profile), a DNA intercalator used in cancer chemotherapy, and its chemical nearest neighbor Carteolol (CID 2583, red line in transporter profile), a non-selective beta blocker used to treat glaucoma, are not actually quite structurally similar (Tanimoto coefficient=0.64) and have significantly different logBB values. After including the transporter descriptors, Doxorubicin and its new nearest neighbor Vincristine (CID 5978, green line in transporter profile), a mitotic inhibitor used in cancer chemotherapy, have closer logBB values. The transporter interaction profiles of these two compounds are similar and they both have higher affinity in four of the five efflux pump transporters (MDR1, MRP1, MRP3, MRP4 and MRP5) than Carteolol. This supports the theory that higher interaction activity with efflux transporters indicates lower BBB permeability. Regarding the two query compounds in group D and E in Table I, their chemical nearest neighbors have same logBB values but the model predictions are still not close. This is due to the existence of other chemically similar compounds with different logBB values that were used to predict the activities of the query compounds. The query compounds have higher similarities in the transporter interaction profile with their new neighbors after including the transporter descriptors (Table I). The better predictions benefit from increasing the distance in chemical space for the structural nearest neighbors with different transporter interaction profiles as well as decreasing distance for those with similar transporter interaction profiles. In group F and G, specifically, the combined evaluation results in the same nearest neighbors, thus the third line in each of these groups showed the second combined nearest neighbor. The correction of prediction is not from the first nearest neighbor itself, but from a combination of neighbors. The first nearest neighbors in these cases have “activity cliffs” with the query compounds, and the next neighbors help minimize the prediction error. The limitation of transporter assays to clear “activity cliffs” also suggests the limitation of information provided by the nine transporter assays.

Using our in-house automatic profiling tool (9), 310 PubChem assays were identified to have data for the compounds in our BBB permeability dataset. Among them, 155 PubChem assays were identified to be somewhat correlated with the BBB permeability with Predictivity 70% or Predictivity 30% (Fig. 5). Among these PubChem assays, many of the assay targets and receptors were proven to regulate, be regulated by or be relevant to BBB permeability, *e.g.*, androgen receptor (40), MDR1 (31), serotonin (5-HT) receptor (41), adenylate cyclase (42), etc. See Supplementary file Table SIII for those assays and description. For example, for PubChem assays correlated to high BBB permeability (144 assays as circled in the orange dots in Fig. 5a), the 35 compounds with the highest BBB permeability (logBB values range from 0.85 to 1.65) show higher P_{sum} values in these assay results than the other compounds with lower BBB permeability. Therefore, if a compound shows an active response in these bio-assays, it is likely to have high BBB permeability. One particular useful assay (AID 943) is a qHTS assay to identify small molecule antagonists of the androgen receptor signaling pathway. Androgen was reported to upregulate the transmembrane transporter MRP4 through androgen receptor activation in prostate cancer cells (43), thus, it was considered as a potentially informative bio-descriptor resource. Among the compounds tested in this assay, the P_{sum} increases with logBB value increment (data not shown). The average values of the 144 positively-correlated assay (as circled in the yellow dots in the bottom of Fig. 5a) results show similar correlation with logBB values (orange line in Fig. 5b) and the average values of the other 11 negatively-correlated assays (as circled in the green dots in the top of Fig. 5a) show reversed correlation with logBB values (green line in Fig. 5b). The remaining assays, identified as non-correlated, (not circled in Fig. 5a) show no/low correlation with logBB values (yellow line in Fig. 5b).

This analysis provided many potential targets as meaningful biological descriptors, but their uses are limited due to missing data. This can be addressed by deriving corresponding individual QSAR models whose predictions are then used as descriptors, or by developing novel algorithms to integrate the currently available assay data into the modeling process. The current hybrid logBB model is expected to be further enhanced when this information is included.

CONCLUSION

In this study, we compiled a large and diverse BBB permeability dataset consisting of 439 unique compounds and applied a consensus QSAR approach to develop predictive logBB models. All of the resulting models showed predictivity that is better than or comparable to those previously reported. The consensus model obtained by averaging the predictions of individual models achieved similar predictivity to the best individual models.

QSAR models for nine transporters were used to generate extra descriptors for the compounds in the BBB permeability dataset. Hybrid models, based on the combination of the same chemical descriptors and nine transporter descriptors, showed better performance than traditional QSAR models. Through analyzing the nearest neighbor compounds in the traditional QSAR and hybrid models, we found that some “activity cliff” issues could be resolved by using hybrid models. Using our in-house automatic profiling tool, some

PubChem assays were also considered to be correlated to BBB permeability. These assays can be potential biological descriptors (after developing their corresponding QSARs) to further improve the current hybrid models. Our research proposed a new strategy to enhance the traditional predictive modeling (*e.g.*, QSAR) of complex biological activities by including extra biological descriptors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

5-HT	5-hydroxytryptamine
AD	Applicability domain
ADME	Absorption, distribution, metabolism, and excretion
AID	PubChem bio-assay identifier
ALDH1A1	Aldehyde dehydrogenase 1 family, member A1
AR	Androgen receptor
ASBT	Apical sodium-dependent bile acid transporter
BBB	Blood–brain barrier
BSEP	Bile salt export pump
CAMP	Cyclic adenosine monophosphate
CID	PubChem compound identifier
CNS	Central nervous system
ER-Alpha	Estrogen receptor alpha
HITs	Human intestinal transporters
HTS	High throughput screening
kNN	k-nearest neighbor
logBB	Logarithm of brain-plasma concentration ratio at steady-state
MAE	Mean absolute error
MCT	Monocarboxylic acid transporters
MDR	Multidrug resistance

MDR1	Multidrug resistance protein 1
MOE	Molecular operating environment software
MRP1	Multidrug resistance-associated protein 1
MRP3	Multidrug resistance-associated protein 3
MRP4	Multidrug resistance-associated protein 4
MRP5	Multidrug resistance-associated protein 5
OATPs	Organic anion transporting polypeptides
PCA	Principle component analysis
QSAR	Quantitative structure-activity relationship
RF	Random forest
SVM	Support vector machine

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Fig. 1. Distribution of compounds by logBB values. *Left (blue)* are “non-permeable” compounds with $\log BB \leq 0$, *right (red)* are “permeable” compounds with $\log BB > 0$.

Fig. 2.
Modeling workflow in this study.

Fig. 3. Chemical space of logBB database ($n=439$) using top three principal components of MOE 2D descriptors (59% variance explained). *Purple dots* are “non-permeable” compounds with $\log BB \leq 0$, *red dots* are “permeable” compounds with $\log BB > 0$.

Fig. 4. Performance of conventional QSAR (based on only MOE descriptors, represented as MOE) and hybrid models (based on both MOE descriptors and transporter assays, represented as HBD) on **a** five-fold cross-validation sets and **b** external set. Last category of each figure is the performance of the consensus model (represented as CSS). Prediction coverage was 100% in all cases.

Fig. 5. The PubChem assay response-BBB permeability correlations: **(a)** Heat-map for the response profiles of 275 compounds against 310 PubChem assays. The assays were sorted by predictivity to BBB permeability, and the AIDs were shown every five assays. The P_{sum} of each assay were calculated for the eight groups consist of 32–37 compounds with similar logBB values within each group. Outlined assays are 11 assays negatively correlated to BBB permeability (circled by *green dots*) and 144 assays positively correlated to BBB permeability (circled by *orange dots*). **(b)** Average P_{sum} values for different PubChem assays with the same compound distribution as above heat-map. (*Orange line*: 144

positively correlated assays, *green line*: 11 negatively correlated assays, *yellow line*: remaining 155 uncorrelated assays).

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Structures	Transporter Profile											
	CID	Exp. logBB	ChemSim	ASBT	MCT1	OATP2B1	BSEP	MDR1	MRP1	MRP3	MRP4	MRP5
2583	0.01	0.64
5978	-1.03	0.61
9864647	0.63	-
10201984	0.88	0.94									

Group C

Query Compound* Predictions:

Pred(MOE) = -0.05

Pred(HBD) = 0.11

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Structures	CID	Exp. logBB	ChemSim	Transporter Profile										
				ASBT	MCT1	OATP2B1	BSEP	MDR1	MRP1	MRP3	MRP4	MRP5
22620091	0.85	0.69
55482	-1.88	-											
	72108	-2	0.8											
	14022522	-1.3	0.64											
	475100	-0.67	-											
Group D Query Compound [*] Predictions: Pred(MOE) = -0.84 Pred(HBD) = -1.13
Group E Query Compound [*] Predictions: Pred(MOE) = -0.43 Pred(HBD) = -0.65

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Structures	Transporter Profile											
	ASBT	MCT1	OATP2B1	BSEP	MDR1	MRP1	MRP3	MRP4	MRP5
-0.67						0.99
-0.23						0.61
-0.98						-		
Group F Query Compound* Predictions: Pred(MOE) = -0.45 Pred(HBD) = -0.6												

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Structures	Transporter Profile											
	ASBT	MCT1	OATP2B1	BSEP	MDR1	MRP1	MRP3	MRP4	MRP5
0.64						0.89
-0.89						0.61
-0.01						-		
<div>Group G Query Compound * Predictions: Pred(MOE) = -0.54 Pred(HBD) = -0.33</div>												

Structures	Transporter Profile											
	CID	Exp. logBB	ChemSim	ASBT	MCT1	OATP2B1	BSEP	MDR1	MRP1	MRP3	MRP4	MRP5
23274095	-0.01	0.84
10352163	-0.24	0.84									

In transporter profiles, blue: query compound, red: chemically nearest neighbor, green: combined nearest neighbor

Abbreviations: *Pred(MOE)* predicted logBB value from model based on the MOE descriptors, *Pred(HBD)* predicted logBB value from the hybrid model, *Exp. logBB* experimental logBB value, *ChemSim* chemical similarity to the query compound

* Query compound, listed as the first compound in each group, was compared to the two neighbors using the chemical w/o transporter descriptors. Second compound in each group is the chemically nearest neighbor. The third compound in group is the top nearest neighbor (in groups A, B, C, F and G) or second nearest neighbor (in groups D and E when the second compound is also the top nearest neighbor) with hybrid descriptors