

RESEARCH ARTICLE

Integrated Application of Enhanced Replacement Method and Ensemble Learning for the Prediction of BCRP/ABCG2 Substrates

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Abstract: Background: Breast Cancer Resistance Protein (BCRP or ABCG2) is a polyspecific efflux-transporter which belongs to the ATP-binding Cassette superfamily. Up-regulation of BCRP is associated to multi-drug resistance in a number of conditions, e.g. cancer and epilepsy. Recent proteomic studies show that high expression levels of BCRP are found in healthy human intestine and at the blood-brain barrier, limiting the absorption and brain distribution of its substrates. Therefore, the early recognition of BCRP substrates seems to be crucial in the early phase of drug discovery.

Objective: The development of computational models that allow the early detection of BCRP substrates and non-substrates.

Method: We have jointly applied the Enhanced Replacement Method and ensemble learning approaches to obtain combinations of 2D linear classifiers capable of discriminating among substrates and non-substrates of the wild type human BCRP.

Results: The ensemble learning approach combining the 10-Enhanced Replacement Method best individual models obtained through MAX Operator displayed the best ability to discriminate between BCRP substrates and non-substrates across all the validation sets/libraries used.

Conclusion: The best model ensemble obtained outperforms previously reported 2D linear classifiers, showing the ability of the Enhanced Replacement Method and ensemble learning schemes to optimize the performance of individual models. This is the first application of the Enhanced Replacement Method to solve classification problems.

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INTRODUCTION

Breast Cancer Resistance Protein (BCRP, also known as ABCG2) is an ATP-dependent efflux pump characterized by broad substrate specificity. It limits the absorption and biodistribution and promotes the elimination of structurally and functionally heterogeneous substrates, including anticancer, antiepileptic, antiviral and antihypertensive drugs, among others. Up-regulation of BCRP expression has been linked to multi-drug resistance issues in many disorders, such as cancer [1-2] and epilepsy [3-5]. Though most of the early research on ATP-binding cassette (ABC) transporters has focused on P-glycoprotein (Pgp, ABCB1), attention has recently been drawn to other members of the superfamily: recent reports demonstrate that BCRP expression levels at both the small intestine and the blood-brain barrier of healthy individuals are

comparable (and even higher) than those of Pgp [6-7], suggesting a prominent role of BCRP in drug pharmacokinetics. Therefore, regulation of BCRP activity and/or recognition of BCRP substrates in the early phase of drug discovery are critical to enhance drug bioavailability and design novel therapeutics aimed at diseases linked to BCRP-mediated multi-drug resistance issues.

To date, limited studies have been reported on the development of high-throughput *in silico* models for the early recognition of BCRP substrates [8-9]. The main limitations of these models are the inclusion of conformation-dependent (3D) molecular descriptors and that they have usually been derived from unbalanced training sets in which substrates are overrepresented in comparison to non-substrates (with the exception of the recent report from Erić and colleagues [10], where a balanced dataset was used). The use of 3D descriptors (which demand conformational pre-optimization of the screened compounds) limits the application of such models in virtual screening campaigns, in which chemical repositories containing thousands to millions of small molecules are typically screened

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through *in silico* filters. On the other hand, inferring models from unbalanced training sets tend to produce predictions that are biased towards the overrepresented category of training examples [11-12].

We have recently reported an ensemble of linear classifiers obtained through Stepwise Forward Linear Discriminant Analysis. These classifiers are entirely based on conformation-independent (0-2D) molecular descriptors and they have been derived from a balanced and representative training set obtained through a clustering procedure [13].

Here, we have resorted to the Enhanced Replacement Method (ERM) [14-15] and ensemble learning in an attempt to improve the performance of the previously reported model-ensemble.

MATERIALS AND METHODS

Dataset Compilation and Partition into Representative Training and Test Sets

The dataset was compiled from literature. For this purpose, we have considered a compound as a substrate only if it is efficiently transported by BCRP and as a non-substrate otherwise. Correspondingly, compounds that bind to BCRP but are not transported are not considered substrates. A 305-compound diverse dataset containing BCRP substrates and non-substrates was initially compiled from 150 articles. From this initial dataset, additional inclusion and exclusion criteria were considered to define the final dataset. Firstly, since it has been reported that single-nucleotide substitutions modify the substrate specificity of BCRP [16-24], and taking into account that the clinical importance of such variants is not clear at the moment [25], we have only kept substrates from wild type BCRP in the final dataset. Secondly, substrates of BCRP homologs from other species with no evidence of human BCRP-mediated transport were not included to avoid noise due to inter-species variability in substrate specificity. As a result, from the initial 305-compound dataset only 262 compounds were kept: 156 substrates and 106 non-substrates of human wild-type BCRP. The dataset was split into a balanced and representative 164-compound training set (consisting in 85 substrates and 79 non-substrates) and a 98-compound independent test set (71 substrates and 27 non-substrates). In order to obtain representative partitions of the dataset, the LibraryMCS v0.7 (ChemAxon, 2011) hierarchical clustering procedure has been jointly applied with the k-means optimization clustering algorithm implemented in Statistica 10 Cluster Analysis Module (Statsoft Inc., 2011). In the recent years, many novel clustering algorithms have been developed for different applications [26-30]. The LibraryMCS is a hierarchical clustering procedure that uses the maximum common substructure (MCS, the largest subgraph found in two chemical graphs) in combination with molecular fingerprints to group a set of small molecules. It has specifically been developed for the clustering of small molecules and it has been widely applied for such purpose [31-34]. Following Everitt *et al.* advice [35], hierarchical clustering was applied to decide on an initial partition of n molecules into k groups; this preliminary clustering was then optimized through the k-means approach, minimizing the

Euclidean distance to the group centers. A number of molecular descriptors calculated with Dragon 4.0 (Milano Chemometrics, 2003) reflecting diverse features of the molecular structure (logP, number of H bonds donors and acceptors, molecular weight, sum of atomic van der Waals volumes, polar surface area, sum of atomic Sanderson electronegativities, 2D Petitjean shape index and total information index of atomic composition) were normalized and used to compute such distance. After the clusters were independently recognized in the substrates and non-substrates categories, around 50% of each cluster from the substrate category and 25% of each cluster in the non-substrate category were randomly assigned to a test set for validation purposes. The remaining elements were kept as calibration set. This scheme provided a balanced training set where neither the substrates nor the non-substrates were overrepresented. Details on the training and test set compounds are available as Supplementary information so that the reader can appreciate the structural diversity of the dataset.

Descriptor Computation and Modeling Procedure

Dragon software v. 6.0 (Milano Chemometrics, 2012) was applied for the computation of 2029 conformation-independent descriptors, distributed along 19 descriptor-blocks, among them topological indices, ring descriptors, 2D atom pairs, information indices and others. A very important issue in multivariate analysis is to remove data having low variance using pre-filtering processes [36-37]. Here we have applied the initial filtering steps provided by Dragon software to exclude molecular descriptors with low information content, including constant or near-constant values (identical values for all the training examples but one) and descriptors with standard deviation below 0.001.

A binary, dummy variable linked to the category of each compound was used as dependent variable (class = 1 for substrates and class = -1 for non-substrates). The ERM [14-15] was used to select, from the descriptor pool, linear combinations of descriptors capable of predicting whether a chemical compound is or is not a BCRP substrate. The original Replacement Method (RM) was developed to explore the descriptor space in an efficient manner, in search of a subset of molecular descriptors from a large set of descriptors [38-40]. It is a rapidly convergent iterative algorithm which produces linear models that perform quite close to the full search solution with much less computational cost. Briefly, an initial subset of descriptors d from the pool of D descriptors is randomly selected. One of these selected descriptors, X_i , is then replaced one by one with all the remaining ($D-d$) descriptors in the pool. The subset of descriptors with the smallest standard deviation (S) is kept. From this resulting subset, the descriptor with the largest standard error in its regression coefficient is substituted one by one with all the remaining ($D-d$) descriptors in the pool. If the replacement of the descriptor with the largest error by those in the pool does not decrease the value of S , then the descriptor is not changed. The procedure is repeated until the selected subset of descriptors remains unmodified. An improvement on the RM, called the Modified Replacement Method (MRM) [15] uses the same strategy except that in each step the descriptor with the

largest error is substituted even when that replacement is not associated to a smaller value of S . The MRM converges to different solutions, commonly bounces from one point to another and simulates 'a higher noise' than the RM, though keeping the overall decreasing trend of the S function. This apparent thermal agitation makes the MRM less likely to get trapped in local minima at the cost of larger computer time. The ERM arises from the combinations of the RM and MRM following the RM-MRM-RM sequence; it combines the good features of both methods and is the only algorithm that goes through a complete simulated annealing cycle [15]. ERM is less dependent on the starting subset of descriptors and shows less propensity to fall in local minima. ERM has also proven to provide better models than the more complex Genetic Algorithms [14].

Here, we have applied the ERM to obtain models including from 4 to 12 molecular descriptors. Models with a larger number of predictors were not considered to maintain the observations to predictor ratio above 15, reducing the chance of overfitting [41-42].

Only those models containing descriptors with significant coefficients at an alpha level of 0.05 were retained. Leave-one-out cross-validation and external validation (discussed in the next section) were used to assess each model's robustness and predictive ability.

External Validation and Model Comparison

The 98-compound independent test set was used for external validation. Later, we have applied Receiving Operating Characteristic (ROC) curves analysis to assess and compare model performance [43]. The ROC curves are graphical plots of Sensitivity (Se) versus 1 minus Specificity (Sp), which provide a rational frame to balance type I and type II errors; they are also useful to optimize a model cutoff score value and to compare models statistically. Se represents the true positives (TP) rate, while Sp refers to the true negatives (TN) rate:

$$Se = \frac{TP}{TP + FN}$$
$$Sp = \frac{TN}{TN + FP}$$

where FP denotes False Positives and FN stands for False Negatives. In our particular application, we are searching for drugs which are not recognized by BCRP (BCRP non-substrates) which will be considered our hits or positives, whereas we want to discard BCRP substrates, which will be then regarded as negatives. A perfect classifier presents an Area Under the Receiving Operating Characteristic curve (AUROCc) of 1, while random classification is associated to an AUROCc of 0.5. MedCalc (MedCalc software, 2011) was used to obtain and statistically compare ROC curves. For statistical comparison of two AUROCc, the nonparametric method of DeLong *et al.* [44] was used for the calculation of the standard error of each AUROCc and then the z-statistic was calculated in order to obtain the correspondent p-value [45].

It has been observed that standard errors of enrichment metrics such as the AUROCc used here are higher for small

datasets than for large datasets [46]. Moreover, using a limited test set with a relatively high proportion of positives leads to a saturation effect: once the hit compounds saturate the early part of the ranking the enrichment metric cannot get any higher. If a model is conceived to analyze large chemical repositories where very few hits might be dispersed among a large number of non-hits, a more challenging and informative test is to conduct a pilot screening on a large database in which relatively few hits are dispersed among a high proportion of non-hits. Therefore, we have built two pilot chemical libraries in order to estimate in a more realistic way the utility of our model in a real virtual screening setting.

On the one hand, we built a 577-compound pilot chemical library (called simulated library) in which our 98-compound test set was dispersed among 479 putative BCRP substrates acting as decoys. Since the human BCRP substrates reported in literature are limited, our putative substrates are substrates of non-human BCRP homologs from other species or highly similar compounds to known human BCRP substrates retrieved from ZINC and PubChem databases through molecular similarity searches (similarity score > 0.75 compared to known substrates). The resulting pilot library contains 27 known non-substrates among 550 known or putative substrates, leading to a hit ratio smaller than 0.05. The same pilot library has previously been applied to assess the performance of our already reported classificatory models (which were derived from the same training set through Stepwise Forward Linear Discriminant Analysis) [13].

As a final challenge to our models, we have used the Enhanced Directory of Useful Decoys resource (DUD-E) [47-48] to build a second, larger and more diverse chemical library (which will be called DUD-E library) containing 1346 compounds (1248 decoys plus the original 98-compound test set) where each decoy is physicochemically similar but topologically dissimilar to its corresponding non-substrate. For this purpose we used the automated decoy generation tool available online. Succinctly, the decoys are property-matched to known hits (in our case, the known non-substrates) using molecular weight, an estimated LogP (miLogP), hydrogen bond donors and acceptors count, rotatable bonds and net molecular charge. About 50 decoys for each compound are chosen from ZINC [49] using a dynamic protocol that adapts to the local chemical space by narrowing or widening windows around the 6 properties. The goal is to obtain from ZINC 3000 to 9000 potential decoys matching the compounds. In a final step, ECFP4 fingerprints are generated for the compounds and its potential decoys; each compound's decoys are ranked by their maximum Tanimoto coefficient and the most dissimilar 25% are kept.

Ensemble Learning

Ensemble learning uses multiple learning algorithms to obtain better predictive performance than the one that could be obtained from any of the individual constituent learning algorithms [50]. Here we have combined a) the scores of the 10 individual models that displayed the best AUROCc for the test set and; b) the scores of all the individual models that showed a global accuracy above 70% for the test set (in total, 97 models). We have used five combination schemes to

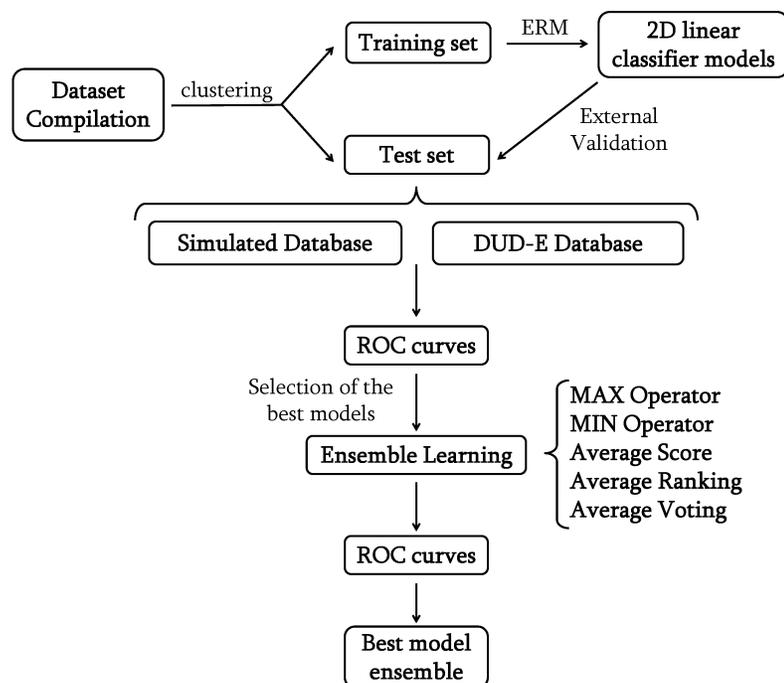


Fig. (1). Schematic representation of the modeling procedure.

obtain a combined score: MAX Operator; MIN Operator; Average Score; Average Ranking and; Average Voting. Voting was computed according to the equation previously used by Zhang and Muegge [51]: the vote obtained by the j^{th} compound in the i^{th} model is equal to $\max(0, \text{int}(11 - \text{rank}_{ij}/0.02\text{NDB}))$, where rank_{ij} is the ranking of the j^{th} compound according to the i^{th} model, and NDB is the number of compounds in the entire screened library. This procedure gives 10 votes to the first 2% ranked compounds, 9 votes for the next 2%, and so on. Compounds in the bottom 80% of the ranking list receive no votes. The five combination schemes were analyzed and compared through ROC curves.

Fig. 1 shows a flowchart summarizing the modeling methodology.

RESULTS AND DISCUSSION

459 models incorporating between 4 and 12 molecular descriptors were obtained through the ERM. All of them displayed good results in the LOO cross-validation. 97 of them showed an overall percentage of good classification above 70% for the test set. The features of the 10 models showing the highest AUROCc for the test set are provided in Table 1. Dragon's nomenclature for the descriptors has been kept. A more detailed insight into the performance of each of these models on the training and test sets, the simulated library and the DUD-E library is presented in Table 2. We have also included the results for the best individual model previously reported [13] (inferred through Stepwise Forward Linear Discriminant Analysis) and the two best 2-model ensembles obtained in that same study. All the individual models showed an AUROCc statistically different from random classification ($p < 0.0001$) for the training set, the

test set and the 577-compound simulated library (Table 2). All the 10 best models obtained through the ERM outperformed the best model obtained through Stepwise Forward Linear Discriminant Analysis (SF) for both the training and test sets, while all models display a similar behavior on the simulated library. Considering the AUROCc for the test set, the best ERM individual model was M438, which slightly outperforms the two best 2-model ensembles obtained through the Stepwise Forward approach (SF-E1 and SF-E2) in relation to the classification of the training and test set compounds; it also slightly outperforms SF-E1 regarding the simulated library classification while it shows similar performance to SF-E2 ($p = 0.6651$). The results suggest that the ERM provides better results than the Stepwise Forward approach. However, all the individual models experimented a significant drop on their performance when they were applied on the DUD-E library, evidently our most challenging test.

The sharp drop in all the individual models performance when applied to the DUD-E library demonstrates the difficulty to find a single linear relationship capable of accurately classifying substrates and non-substrates when facing a real *in silico* screening application. In this sense, one should keep in mind that the broad substrate specificity of BCRP makes our modeling problem particularly challenging. It has been pointed out that the polyspecificity of ABC transporters due to multiple binding sites and high protein flexibility determines a complex phenomenon which can only be partially addressed by current methods in the computational drug design field [52-53]. This explains why many modeling efforts to identify ABC transporters substrates have resorted to ensemble learning or locally weighted methods [54-57]. Thus, we have applied ensemble learning methods combining the best ERM individual

Table 1. Summary of the features of the 10-ERM individual models which showed the best performance on the test set.

Model	Descriptors included	F	Wilk's λ	Overall accuracy Training Set	Overall accuracy Test Set
M36	SM5_B(e): spectral moment of order 5 from Burden matrix weighted by Sanderson electronegativity P_VSA_i_2: P_VSA-like on ionization potential, bin 2 SpDiam_EA: spectral diameter from edge adjacency mat. nRCONHR: number of secondary amides (aliphatic)	19.970	0.666	83.5	75.5
M61	SpMax2_Bh(s): largest eigenvalue n. 2 of Burden matrix weighted by I-state SpMaxA_EA(ed): normalized leading eigenvalue from edge adjacency mat. weighted by edge degree nRCONHR CATS2D_07_NL: CATS2D Negative-Lipophilic at lag 07 F01[C-C]: Frequency of C - C at topological distance 1	19.211	0.622	79	71
M107	EE_B(e): Estrada-like index (log function) from Burden matrix weighted by Sanderson electronegativity P_VSA_i_2 SpDiam_EA nRCONHR B10[C-C]: Presence/absence of C - C at topological distance 10 DLS_01: modified drug-like score from Lipinski (4 rules)	18.373	0.587	83.5	71
M203	IDE: mean information content on the distance equality SpMaxA_Dz(e): normalized leading eigenvalue from Barysz matrix weighted by Sanderson electronegativity SpPosA_B(v): normalized spectral positive sum from Burden matrix weighted by van der Waals volume nArOR: number of ethers (aromatic) S-108: R=S NaaaC: Number of atoms of type aaaC CATS2D_07_NL B10[C-C]	18.426	0.512	83	70
M289	N%: percentage of N atoms SpMax2_Bh(s) SM11_EA: spectral moment of order 11 from edge adjacency mat. Eig05_EA: eigenvalue n. 5 from edge adjacency mat nC_s: number of total secondary C(sp3) nRCONHR NaaaC CATS2D_07_NL Ui: unsaturation index	17.838	0.490	83.5	74.5
M356	N% nR04: number of 4-membered rings SpMax2_Bh(s) SM11_EA SM13_AEA(bo): spectral moment of order 13 from augmented edge adjacency mat. weighted by bond order nC_s nRCONHR NaaaC CATS2D_07_NL Ui	17.841	0.462	85	73.5

Table 1. (contd....)

M380	<p>IDE</p> <p>WiA_Dz(m): average Wiener-like index from Barysz matrix weighted by mass</p> <p>SpPosA_B(v)</p> <p>SpMax2_Bh(s)</p> <p>SaaC: Sum of aaaC E-states</p> <p>SdS: Sum of dS E-states</p> <p>CATS2D_05_DA: CATS2D Donor-Acceptor at lag 05</p> <p>CATS2D_01_NL: CATS2D Negative-Lipophilic at lag 01</p> <p>B10[C-C]</p> <p>DLS_01</p>	17.415	0.468	85	73.5
M438	<p>nR07: number of 7-membered rings</p> <p>D/Dtr04: distance/detour ring index of order 4</p> <p>piPC09: molecular multiple path count of order 9</p> <p>ATSC2e: Centred Broto-Moreau autocorrelation of lag 2 weighted by Sanderson electronegativity</p> <p>GATS1e: Geary autocorrelation of lag 1 weighted by Sanderson electronegativity</p> <p>SpMax2_Bh(i): largest eigenvalue n. 2 of Burden matrix weighted by ionization potential</p> <p>SpMax2_Bh(s)</p> <p>SM04_EA(ed): spectral moment of order 4 from edge adjacency mat. weighted by edge degree</p> <p>Eig10_EA(dm): eigenvalue n. 10 from edge adjacency mat. weighted by dipole moment</p> <p>Uc: unsaturation count</p> <p>DLS_01</p>	17.588	0.440	86	75.5
M446	<p>nR04</p> <p>SpMax_A: leading eigenvalue from adjacency matrix (Lovasz-Pelikan index)</p> <p>ATSC2e</p> <p>SpMax2_Bh(s)</p> <p>P_VSA_i_2</p> <p>Eig05_EA</p> <p>nRCONHR</p> <p>NaaaC</p> <p>B03[O-O]: Presence/absence of O - O at topological distance 3</p> <p>F09[C-S]: Frequency of C - S at topological distance 9</p> <p>cRo5: Complementary Lipinski Alert index</p>	17.419	0.442	85	71
M471	<p>SRW09: self-returning walk count of order 9</p> <p>IDE</p> <p>WiA_Dz(e): average Wiener-like index from Barysz matrix weighted by Sanderson electronegativity</p> <p>SpMax2_Bh(s)</p> <p>Eta_betaS_A: eta sigma average VEM coun</p> <p>C-034: R--CR..X</p> <p>CATS2D_08_DP: CATS2D Donor-Positive at lag 08</p> <p>CATS2D_07_NL</p> <p>B02[C-O]: Presence/absence of C - O at topological distance 2</p> <p>B08[N-O]: Presence/absence of N - O at topological distance 8</p> <p>B10[C-C]</p> <p>Ui</p>	18.666	0.403	88	74.5

Table 2. *Se*, *Sp*, Global accuracy and AUROCc values of the 10 best individual models obtained with the ERM for the training and test sets, the simulated library and the DUD-E library. Such values for the best previously reported individual model (SF) and the two best 2-model ensembles (SF-E1 and SF-E2) obtained through the Stepwise Forward technique have also been included for comparison purposes. A score of zero has been considered as the cutoff value to assess *Sp* and *Se*. The best AUROCc for each set of compounds has been indicated in bold.

Model	Training Set				Test Set				Simulated Library				DUD-E Library			
	<i>Sp</i>	<i>Se</i>	Global	AUROCc	<i>Sp</i>	<i>Se</i>	Global	AUROCc	<i>Sp</i>	<i>Se</i>	Global	AUROCc	<i>Sp</i>	<i>Se</i>	Global	AUROCc
M438	91	81	86	0.945**	85	52	75.5	0.823**	76	52	74.5	0.755**	51.8	61.6	61.4	0.503
M380	86	85	85	0.939**	72	78	73.5	0.789**	68	78	67	0.746**	77.8	57.5	57.9	0.576
M107	82	85	83.5	0.894**	73	67	71	0.788**	61	67	61	0.719**	66.7	48.6	49	0.636*
M471	86	90	88	0.957**	76	70	74.5	0.788**	75	70	74.5	0.765**	70.4	45.4	45.9	0.611*
M356	87	84	85	0.946**	79	59	73.5	0.783**	70	59	69.5	0.747**	59.3	51	51.2	0.591
M61	86	71	79	0.885**	75	63	71	0.774**	70	63	67	0.756**	63	46.7	47	0.666*
M203	82	84	83	0.924**	70.4	70.4	70	0.774**	67	70.4	71	0.736**	70.4	57.4	57.6	0.590
M289	85	82	83.5	0.936**	59	80	74.5	0.774**	71.5	80	69.5	0.751**	59.3	50.1	50.3	0.580
M36	88	78	83.5	0.863**	74	76	75.5	0.773**	65	76	66	0.725**	74.1	47.4	47.9	0.634*
M446	86	85	85	0.948**	77.5	55.5	71	0.772**	66	55.5	65	0.732**	55.5	51.1	51.2	0.610†
SF	79	68	74	0.796**	63	74	66	0.748**	66	74	66	0.732**	46	74.1	46.6	0.622†
SF-E1	84	75	79	0.850**	70	74	71	0.785**	64	74	64	0.736**	53.6	74.1	54.1	0.660*
SF-E2	85	80	82	0.902**	76	70	74.5	0.804**	68	70	68	0.771**	48	70.4	48.5	0.637†

AUROCc statistically different from a random classification (AUROCc = 0.5) ** p < 0.0001; * p < 0.01, † p < 0.05

Table 3. The table shows the AUROCc values for the 10- and 97-ERM model ensembles obtained through the five combination schemes for the training and test sets, the simulated library and the DUD-E library; the AUROCc values for the best individual model M438 have also been included. The best AUROCc for the 10-model ensemble has been highlighted in bold for each set of compounds.

10-model ensemble	AUROCc			
	Training Set	Test Set	Simulated Library	DUD-E Library
MAX Operator	0.954**	0.850**	0.824**	0.743**
MIN Operator	0.939**	0.776**	0.724**	0.526
Average Score	0.963**	0.833**	0.791**	0.628*
Average Ranking	0.963**	0.830**	0.789**	0.629†
Average Voting	0.871**	0.724**	0.711**	0.558
97-model ensemble	Training Set	Test Set	Simulated Library	DUD-E Library
MAX Operator	0.829**	0.647†	0.530	0.589
MIN Operator	0.934**	0.740**	0.573	0.512
Average Score	0.964**	0.778**	0.745**	0.598
Average Ranking	0.963**	0.781**	0.745**	0.591
Average Voting	0.906**	0.712**	0.651*	0.622†
M438	0.945**	0.823**	0.755**	0.503

AUROCc statistically different from a random classification (AUROCc = 0.5) ** p < 0.0001; * p < 0.01, † p < 0.05

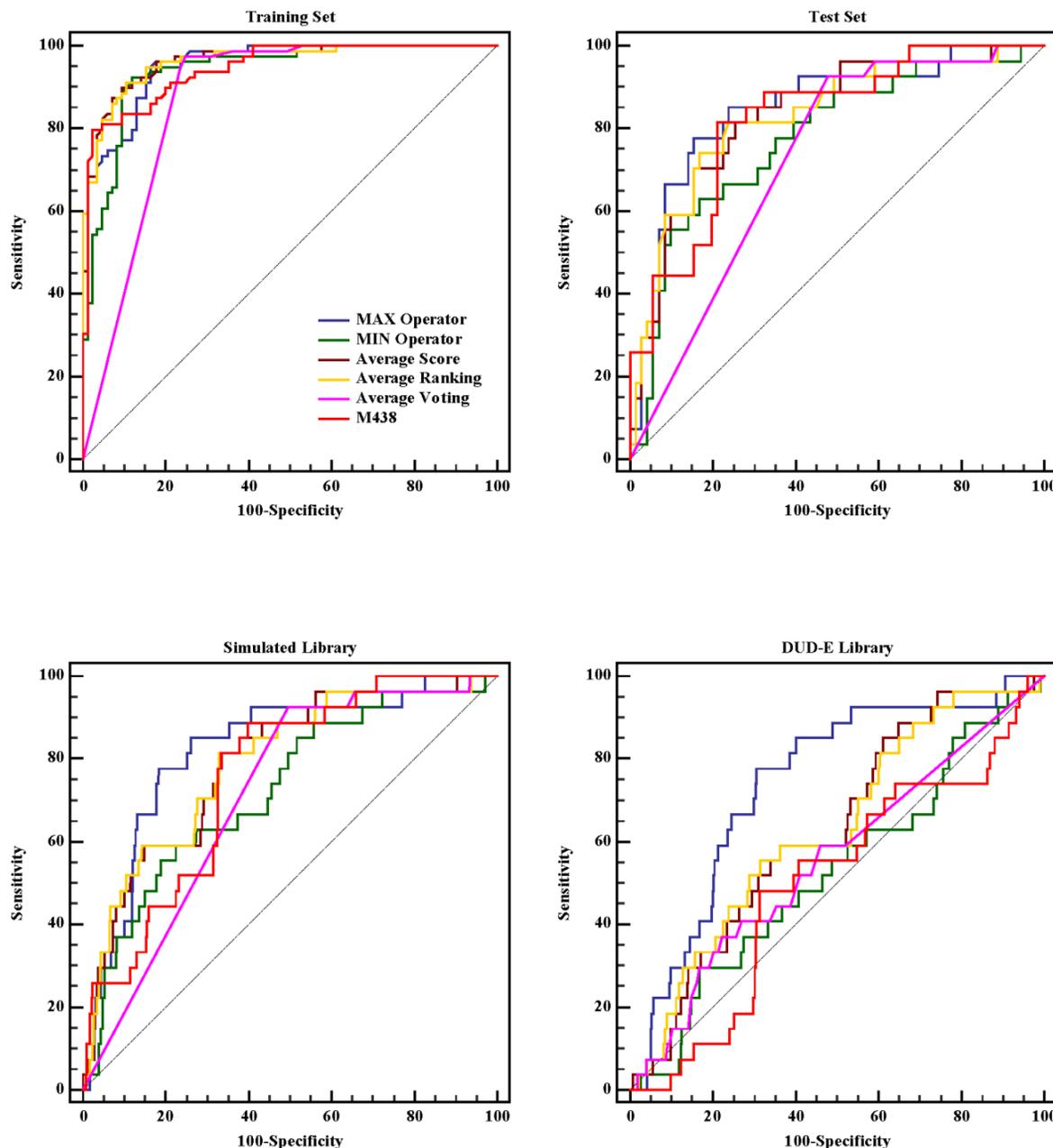


Fig. (2). Graphical comparison of the ROC curves from the best performing ensembles and the best ERM individual model (M438) for each set of compounds assessed. Selective ensemble of 10 models through the MAX Operator is the only approach that consistently shows good performance on all the validations sets/libraries used.

models through five different combination schemes, leading to remarkably improved results (Table 3; the best ERM individual model –M438– has been included in the table to facilitate the comparison).

Compared to the best ERM individual model, the combination of the 10 best individual ERM models using MAX Operator showed very similar performance on the training and the test sets ($p = 0.5626$ and $p = 0.4522$, respectively), but it markedly improved the performance on the simulated library ($p = 0.0353$) and the DUD-E library (p

< 0.0087). No improvement compared to the best individual model (and, in fact, reduced accuracy) was observed when combining the 97 models that showed best performance on the test set. This suggests that, when resorting to ensemble learning, selective combination of relatively few good models might be better than combining several weak models. This is in agreement with previous studies applying ensemble learning that suggest that the combination of a few and selected classifiers (selective ensemble) may provide better accuracy and generalization than combining all

available learners [58-60]. Based on the previous results, we have also tested combinations of the 2, 3, 5, and 15 models (data not shown) with the best AUROCc on the test sets. None of these ensembles showed statistically significant differences on the training set, the test set and the simulated library AUROCc. The 10-model ensemble displayed the best behavior on the DUD-E library, outperforming the 2-, 3- and 5-model ensembles ($p < 0.0001$) and showing very similar performance to the 15-model ensemble ($p = 0.6679$), constituting the best ensemble obtained.

It is also interesting that, for both the individual models and the model ensembles (no matter which combination scheme was used), the performance on the 98-compound test set is generally better than the performance on the 577-compound simulated library, falling sharply on the 1346-compound DUD-E library. On the one hand, this seems to confirm that assessing the model's performance on large chemical libraries is a more stringent test than assessing performance on small compound sets, and the ability of the Enhanced Directory of Useful Decoys to generate suitable decoys for validation purposes, on the other. The MAX Operator displayed the best results to combine individual learners, with an AUROCc of 0.824 on the simulated library and of 0.743 on the DUD-E library, being the only ensemble scheme that showed a good performance on the larger and more diverse library tested, while all other individual models and model ensembles showed either poor or no classificatory power at all on this library. Fig. 2 shows the ROC curves of the 10-ERM model ensembles and the best ERM individual model for each set of compounds evaluated.

CONCLUSIONS

Using the ERM to deal with a classification problem for the first time, we have developed linear model ensembles capable of identifying BCRP substrates and non-substrates; these models are entirely based on conformation-independent descriptors, thus being capable of screening large chemical repositories with high throughput. It should be highlighted that systematic comparisons of different virtual screening approaches with different levels of sophistication have surprisingly shown that the less sophisticated approaches might sometimes outperform more complex ones in terms of enrichment metrics: more complex methods are not unequivocally better; which method is more suitable seems to be highly target-dependent [51, 61]. Simpler approaches are, however, always more efficient.

The models reported here might be efficiently apply at an early stage in drug discovery projects to discard drug candidates predicted as BCRP substrates, which might present BCRP-associated bioavailability issues, drug-drug interactions and multi-drug resistance issues linked to BCRP. The models reported here clearly outperform previously reported linear classifiers by our group, which were obtained through the simpler Stepwise Forward Linear Discriminant Analysis, and confirm the utility of the ERM to establish useful QSAR relationships. The ensemble learning approach combining the 10-ERM best individual models obtained through MAX Operator displayed the best capacity to discriminate between BCRP substrates and non-substrates across all the validation sets/libraries used here, proving to be effective to improve the predictive ability of the

individual models. These results seem to confirm the strength of the selective ensemble approximation. The sharp drop in the classificatory power of the individual models and model ensembles when studying the DUD-E library underlines the ability of the Enhanced Directory of Useful Decoys to provide challenging collections of small molecules in order to assess computational models' predictive ability previous to proceed to real *in silico* screening applications. Furthermore, it was confirmed that assessing model performance on a large and diverse database is a more stringent test than assessing performance on smaller libraries, allowing estimating in a more realistic way the utility of a model in a real virtual screening setting.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to the present article.

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