Application of Machine Learning Approaches to Identify New Anticonvulsant Compounds Active in the 6 Hz Seizure Model

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Abstract. Epilepsy is the second most common chronic brain disorder, affecting 65 million people worldwide. According to the NIH's Epilepsy Therapy Screening Program, evaluation of potential new antiepileptic drug candidates begins with assessment of their protective effects in two acute seizure models in mice, the Maximal Electroshock Seizure test and the 6 Hz test. The latter elicits partial seizures through an electrical stimulus of 44 mA, at which many clinically established anti-seizure drugs do not suppress seizures. The inclusion of this "high-hurdle" acute seizure assay at the initial stage of the drug identification phase is intended to increase the probability that agents with improved efficacy will be detected. In this work, we have used machine learning approximations to develop *in silico* models capable of identifying novel anticonvulsant drugs with protective effects in the 6 Hz seizure model. Linear classifiers based on Dragon conformation-independent descriptors were generated through an in-house routine in R environment and validated through standard validation procedures. They were later combined through different ensemble learning schemes. The best ensemble comprised the 29 best-performing models combined using the MIN operator. With the objective of finding new drug repurposing opportunities (i.e. identifying second or further therapeutic indications, in our case anticonvulsant activity, in existing drugs), such model ensemble was applied in a virtual screening campaign of DrugBank and Sweetlead databases. 28 approved drugs were identified as potential protective agents in the 6 Hz model. The present study constitutes an example of the use of machine learning approximations to systematically guide drug repurposing projects.

Keywords: Machine learning · Ensemble learning · 6 Hz seizure model · Anticonvulsant drugs · Virtual screening · Epilepsy · Drug repurposing

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1 Introduction

Epilepsy is the second most common chronic brain disorder characterized by recurrent and spontaneous seizures, that affect 65 million all ages people in the world [1]. Around thirty percent of epileptic patients do not respond to clinically established anticonvulsants, a condition known as refractory or intractable epilepsy [2].

Drug repositioning represents an interesting strategy to expedite the development of new medications [3]. This approach consists in searching second or further medical uses for experimental, approved, discontinued and shelved drugs. Computer-aided drug repurposing provides a rational framework to identify repurposing opportunities with minimal investment of time and resources [4].

The 6 Hz psychomotor seizure model of partial seizures in mice uses electrical stimulation by low-frequency (6 Hz) rectangular pulses of 0.2-ms duration delivered through corneal electrodes for 3 s [5], to induce seizures that are reminiscent of the psychomotor ones occurring in human limbic epilepsy. It is currently included in the initial phase of drug screening of the NIH's Epilepsy Therapy Screening Program (ETPS) [6].

This model has been able to identify drugs that are not active in conventional animal models, such as levetiracetam (which has a novel mechanism of action and is effective in treating a wide range of seizures including partial onset refractory epilepsy, a type of epilepsy usually difficult to control by drug therapy). It has been thus suggested as a screening tool to evaluate new antiepileptic drugs (AEDs) with potential activity against refractory seizures [7].

In this work, we have developed *in silico* models capable of discriminating active from inactive compounds in the 6 Hz model. Such models have been then applied in a virtual screening campaign oriented to find drug repurposing opportunities.

2 Methods

2.1 Data Set Compilation and Splitting

90 compounds evaluated in the 6 Hz seizure model were compiled from literature [8–42] and conformed a balanced dataset which includes 44 active compounds (>75% protection at doses ≤ 30 mg/kg) and 46 inactive compounds (no protection up to doses of 100 mg/kg). This dataset was later used to obtain training and test sets to calibrate and validate the models, respectively. It was curated using Standardizer Instant JCHEM v. 16.9.12.0. The molecular diversity of the dataset (both within and between the Active and Inactive classes) can be appreciated in the heatmaps of Fig. 1, which shows, for each pair of compounds in the dataset, the Tanimoto distances calculated using extended connectivity fingerprints with a maximum diameter of 4 (ECFP_4). Such heatmaps were built using Gitools v. 2.3.1 [43] and Tanimoto distances were calculated employing ScreenMD - Molecular Descriptor Screening v. 5.5.0.1. (ChemAxon 2011).

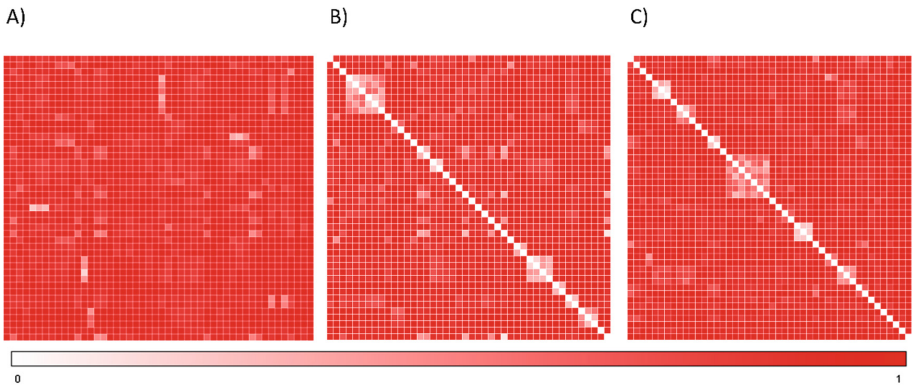


Fig. 1. Heatmaps of dissimilarity for the whole database and within each database category. (A) Active compounds vs. inactive compounds; (B) active compounds vs. active compounds; (C) inactive compounds vs. inactive compounds.

Following previous studies suggesting that rational sampling of the training and test sets lead to models of enhanced predictivity [44, 45] we chose to resort to a clustering approach to partition the dataset representatively. We used a combined hierarchical and non-hierarchical procedure [46]: the LibraryMCS v16.10.10.0 (ChemAxon 2016) hierarchical clustering approach based in the Maximum Common Substructure (MCS) was first applied, and the resulting clusters were then optimized using the k-means algorithm (Statistica 10 Cluster Analysis Module, Statsoft Inc. 2011). Such combined clustering procedure was performed in an independent manner for the Active and Inactive classes to obtain a balanced training set (30 active compounds and 30 inactive compounds). The remaining compounds were assigned to the test set (14 active and 16 inactive compounds). The molecular structures of the training and test set compounds are presented as Supplementary Material.

2.2 Descriptor Calculation and Modelling

Molecular descriptors are numerical variables that reflect different aspects of the molecular structure. The values of 3668 conformation-independent (0D–2D) descriptors were computed with Dragon 6.0 software. A random subspace approach [47, 48] was then used to explore the descriptor space: 1000 random subsets of 200 descriptors each were generated, and one model was trained from each subset. The random subspace approximation causes the models not to over-focus on features that display high explanatory power in the training set. It can also be useful when handling datasets that suffer from small sample size and large dimensionality (i.e. large feature space) (a quite frequent scenario in the drug discovery field) and when the feature space includes redundant features.

A binary variable associated to each dataset class (active and inactive compounds) was used as dependent variable. Such variable was assigned observed values of 1 for compounds within the Active class and observed values of 0 for compounds in the Inactive class. A semi-correlation approach using a Forward Stepwise feature selection

procedure was used to obtain one model from each of the random feature subsets [49]. A tolerance value of 0.5 was selected to exclude highly correlated descriptors from the models. A minimum ratio of 10 between the number of training set examples and the number of descriptors was used in order to reduce the chances of overfitting.

An in-house script in R environment was used for all data analysis. The R package data table (https://cran.r-project.org/package=data.table) was used to handle datasets.

Standard validation approaches, including stratified Leave-Group-Out cross validation, randomization test and external validation, were applied to assess the models' robustness and predictive ability.

Regarding the Leave-Group-Out cross-validation, in each cross-validation round, random subsets comprising 5 active and 5 inactive compounds were removed from the training set, and the model was regenerated using the remaining compounds as training examples. The resulting model was used to predict the class label for the 10 removed compounds. The procedure was repeated 10 times, removing each of the training set compounds at least once. The results were informed as the average percentage of good classifications (accuracy) across the folds, and this was compared with the accuracy of the model for the original training set and also, as advised by Gramatica [50], with the No-Model error rate or risk (NOMER%), i.e. the error provided in absence of model.

In the case of randomization, the class label was randomized across the compounds in the training set. The training set with the randomized dependent variable was then used to train new models from the descriptor selection step. Such procedure was repeated 10 times within each descriptor subset and the average accuracy and the 95% confidence interval for the accuracy of the randomized models were calculated. It is expected that the randomized models will perform poorly compared to the real ones.

At last, the predictivity of each individual model was assessed through external validation, using the 30-compound test set. A diversity of statistical parameters commonly used to assess the performance of classificatory models [50, 51] were estimated for both the training and test sets: sensitivity (Se, i.e. true positive rate), specificity (Sp, i.e. true negative rate), accuracy (Acc. overall percentage of good classifications), positive and negative predictivity and the F-measure.

2.3 Ensemble Learning

An ensemble of classifiers is a set of classifiers whose individual decisions are combined in some way (e.g. through voting, or averaging their scores) to classify new examples; interestingly, ensembles are often much more accurate and provide better generalization than the individual classifiers [47, 52]. There are different reasons that explain the enhanced performance and predictivity of model ensembles [52]. The first is statistical: a learning algorithm can be viewed as a search in a hypothesis space to identify the best hypothesis in it. The learning algorithm can find several different hypotheses that all give the same or similar accuracy on the training data; by constructing an ensemble out of all (or some) of these accurate classifiers, the algorithm can average their votes or score and reduce the risk of choosing the wrong one. A second reason is computational: many learning algorithms work by performing some form of local search that might get stuck in local optima. An ensemble built by running the local search from several different starting points provides a better approximation to

the true (unknown) function than any of the individual classifiers. There are many methods for constructing ensembles, but essentially they comprise enumerating and weighting all possible hypotheses, manipulating the training examples (as in bagging), manipulating the input features (as in the already described random subspace approach), manipulating the output targets (as in boosting) and injecting randomness.

As described below, two retrospective virtual screening campaigns were used to evaluate the performance of individual models and model ensembles. The first retrospective experiment enabled evaluating the performance of individual models and bestow the basis to decide which individual classifiers would be selectively combined in the model ensemble and what operator would be used to combine them. The second retrospective virtual screening was used to validate the performance of the chosen model ensemble.

The best individual classifiers were selected and combined using the area under the ROC curve metric (AUCROC) in the first retrospective virtual screening experiment as criterion of performance. Systematic combinations of the 2–100 best performing classifiers were analyzed. Four combination schemes were applied to obtain a combined score (Fig. 3): MIN operator; Average Score; Average Ranking and Average Voting. AUCROCs were obtained with the pROC package [53]; the DeLong method was used to obtain 95% confidence intervals. BEDROC and RIE (1%) were also computed [54]. For that purpose, we have resorted to the R package enrichvs (enrichment assessment of virtual screening approaches) [55] and the online tool ROCKER [56].

2.4 Retrospective Virtual Screening

Truchon and Bayly [54] demonstrated that the AUCROC metric is dependent on the ratio of actives/inactives, and its standard deviation converges to a constant value when small yield of actives (Ya) of the screened library are used (Ya below 0.05 seems to provide more robust results). A reasonably small Ya also ensures that the saturation effect is constant or absent. A high number of decoys (1000 or higher) and a small Ya contribute to a controlled statistical behaviour. Consequently, we performed retrospective virtual screening campaigns to better estimate the enrichment provided by the individual models and the model ensembles. For this, we have dispersed 14 active compounds from the test set among a large number of paired decoys (putative inactive compounds) provided by the enhanced Directory Useful Decoys (DUD-E) [57]. The first library subjected to retrospective virtual screening consisted in the 14 active compounds with 700 presumed inactive ones (decoys). This library has been denominated DUD-E A library. The second library subjected to retrospective virtual screening is comprised by the 14 active compounds plus 3500 decoys and was named DUD-E B library. DUD-E A was used to estimate the performance of the individual models in a true virtual screening experiment and to train the ensemble (i.e. to decide which individual models would be included and how they would be combined). DUD-E B was only used to validate the performance of the best model ensemble.

2.5 Building Positive Predictive Value Surfaces and Choosing an Adequate Score Threshold Value

A practical concern when implementing *in silico* screening campaigns is to predict the actual probability that a hit will confirm its predicted activity when submitted to experimental testing (Positive Predictive Value, PPV). Estimation of such probability is however precluded due to its dependency on the Ya of the screened library, which is not known *a priori* (Eq. 1):

$$PPV = \frac{SeYa}{SeYa + (1 - Sp)(1 - Ya)} \tag{1}$$

Equation (1) was applied to build PPV surfaces. In order to choose an optimal score cutoff value to select predicted hits in prospective virtual screening experiments, 3D plots illustrating the interplay between PPV, the Se/Sp ratio and Ya were built for each individual model and for each model ensemble [58]. Using the DUD-E A and DUD-E B databases, Se and Sp were computed in all the range of possible cutoff score values. Since controlled statistical behavior is observed for database sizes of about 1000 compounds or more and Ya below 0.05, we can reasonably assume that the ROC curve and derived metrics will be similar when applying the models to classify other large chemical databases with low Ya. Taking into consideration that in real virtual screening applications Ya is ignored a priori but invariably low, Ya was varied between 0.001 and 0.010. The R package plotly (https://cran.r-project.org/package=plotly) was used to obtain all the PPV graphs. Visual analysis of the resulting PPV surfaces allowed us to select a score threshold value with a desired range of PPV.

2.6 Virtual Screening

Based on visual inspections of the resulting of PPV surface graphs, the best model ensemble was used in a prospective virtual screening application. We used a 29-model ensemble using the MIN operator to combine the scores of the individual models, choosing 0.3505 as score threshold. Such threshold corresponds to a Se/Sp ratio of 0.793. It was checked that every hit belonged to the applicability domain of the ensemble model which assigned the minimum score. The leverage approach was used to assess if a hit belongs to the applicability domain, using 3d/n as cutoff value, where d is the number of descriptors in the correspondent model and n is the number of compounds in the training set.

We used the 29-model ensemble to screen two databases: (a) DrugBank 5.0.0, an online database containing extensive information about the US Food and Drug Administration (FDA) approved, experimental, nutraceutical, illicit and investigational drugs [59]; (b) Sweetlead, a curated database of drugs approved by other international regulatory agencies, compounds isolated from traditional medicinal herbs, and regulated chemicals [60]. Both databases were curated using Standardizer version 16.9.12.0 (ChemAxon 2016). We applied the following actions to generate homogeneous representations of the molecular structure for the virtual screen: (1) Strip salts; (2) Remove Solvents; (3) Clear Stereo; (4) Remove Absolute Stereo; (5) Aromatize; (6) Neutralize;

(7) Add Explicit Hydrogens; and (8) Clean 2D. Additionally, duplicated structures were removed using Instant JCHEM v.17.2.6.0.

The general protocol used for building and validating our individual models and model ensembles has been included in Fig. 2.

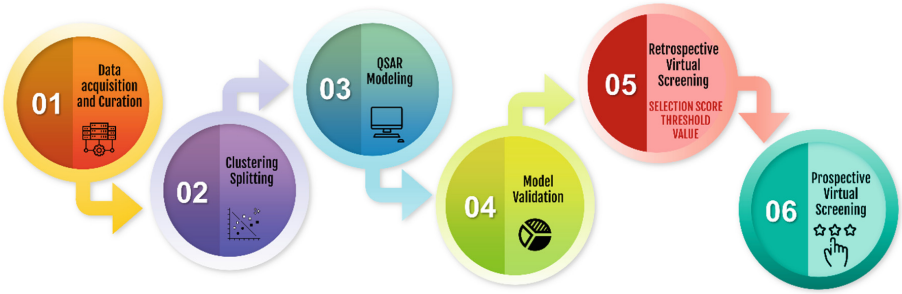


Fig. 2. Computational flowchart for cheminformatics analysis using ensemble learning

3 Results

Ligand-Based Model Development, Validation and Virtual Screening

We resorted to a computationally inexpensive (conformation-independent) ligand-based approach to obtain 1000 individual linear classifiers by applying a random subspace approximation. The individual models were externally validated by using an independent test set and, for a more realistic performance assessment, by retrospective screening of pilot databases, where a small proportion of known active compounds was dispersed among a high number of decoys (putative inactive compounds). Table 1 shows the enrichment metrics of the five individual classifiers that displayed the best performance on the training set, test set and DUD-E A library.

Table 1. Values of the AUCROC metric for the best five individual models.

Model	Training set	Test set	DUDE-A	DUDE-B
191	**0.790 (± 0.0570)**	**0.690 (± 0.100)**	**0.855 (± 0.0426)**	**0.829 (± 0.0460)**
208	0.886 (± 0.0405)	0.884 (± 0.0598)	0.844 (± 0.0397)	0.823 (± 0.0434)
586	0.787 (± 0.0580)	0.862 (± 0.0694)	0.837 (± 0.0521)	0.830 (± 0.0518)
051	0.851 (± 0.0493)	0.871 (± 0.0694)	0.836 (± 0.0507)	0.818 (± 0.0538)
902	0.770 (± 0.0405)	0.786 (± 0.0841)	0.835 (± 0.0372)	0.801 (± 0.0411)

The best individual model included the following features:

Model 191: **Class** = 0.46645 - 0.71571 * **B02[O-O]** - 0.24724 * **CATS2D_00_PP** + 0.02305 * **SM13_EA (dm)**

$F_{(3,56)} = 7.821188$ $p < 0.0002$

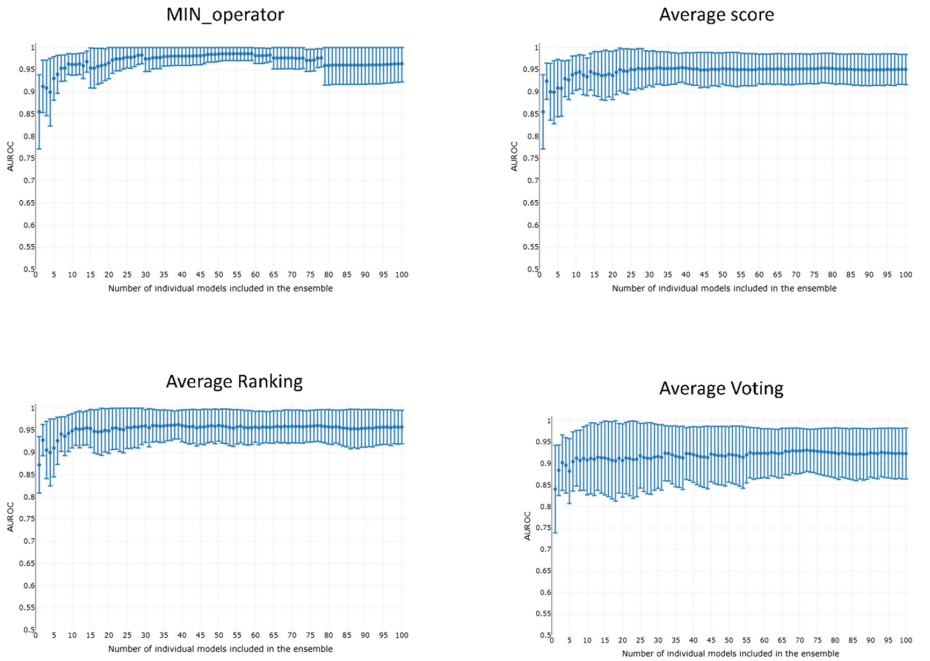


Fig. 3. AUCROC metric vs. the number of combined models in the DUDE-A database (a) MIN operator; (B) Average score; (C) Average ranking; (D) Average voting.

Dragon's nomenclature for the molecular descriptors has been kept in the previous expression. **B02[O-O]** represents the presence/absence of an O-O pair at topological distance 2; **CATS2D_00_PP** refers to the CATS (Chemically Advanced Template Search) 2D Positive-Positive at lag 00; and **SM13_EA (dm)** corresponds to the spectral moment of order 13 from edge adjacency matrix, weighted by dipole moment.

Whereas the performance of the best individual classifiers was acceptable, we resorted to ensemble learning to obtain meta-classifiers with improved accuracy, enhanced enrichment parameters and a more robust behavior. The expectations on the model combination approach were confirmed statistically, showing clear statistical differences in comparison to the best individual model. Figure 3 shows the influence of the number of models included in the ensemble on the AUCROC metric.

The MIN, RANKING and AVERAGE combination schemes exhibited similar classificatory ability and enrichment behavior on the test set, DUDE-A and DUDE-B libraries.

We chose to move to the prospective virtual screening experiment using the ensemble obtained by combining the 29 best-performing individual models using the MIN operator, which was statistically superior to the best individual model on DUDE-A ($p < 0.01$) and DUDE-B ($p < 0.05$) and provided better results than other combination schemes in terms of the deviation associated to the estimated enrichment parameters. Table 2 shows the enrichment metrics for the 29-model ensembles obtained by different combination schemes.

Table 2. Values of metrics for the 29-model ensemble

	Training set		DUDE-A			DUDE-B		
29-model ensemble	AUCROC	BEDROC	AUCROC	BEDROC	$RIE_{1\%}$	AUCROC	BEDROC	$RIE_{1\%}$
MIN-operator	**0.951**	**0.999**	**0.983**	**0.841**	**36.22**	**0.975**	**0.752**	**64.43**
Average score	0.942	0.998	0.951	0.627	14.49	0.946	0.646	35.80
Average ranking	0.940	0.998	0.959	0.721	28.98	0.938	0.664	50.11
Average voting	0.941	0.998	0.916	0.572	14.49	0.887	0.525	28.64

We decided to optimize the score cutoff value by resorting to analysis of PPV surfaces [57]. With the help of these surfaces, the evolution of the most relevant metric for our purposes, the PPV value, can be visually (or, eventually, mathematically) optimized as a function of the Se/Sp ratio across a range of Ya values. We built such surfaces using the data from the retrospective screening of DUDE-A. The strongest assumption of our approach is that the Se/Sp value observed for a given score during this retrospective screening experiment will hold when screening other databases (e.g., the ones screened in prospective virtual screening applications). This is of course not necessarily true. However, since the AUCROC values obtained for the DUD-E libraries are unequivocally high (always above 0.9 for the individual models and very close to the perfect value of 1 for the ensembles) while on the other hand the DUD-E database Ya ratio (quite below 0.05) and size (>1000 compounds) speak of a controlled statistical behavior, we believe it is a reasonable assumption in the present setting.

Using the PPV surface, we chose 0.35 as score threshold to be used in our virtual screening of DrugBank and Sweetlead; such score is associated to a Se/Sp ratio of 0.793 for the 29-model ensemble based on the MIN operator and to PPV value $\geq 20\%$ for a Ya of 0.01 (Fig. 4). This means that if Ya in the real virtual screen was 0.01, we would have to submit about five predicted hits to experimental testing in order to find one confirmed hit. The virtual screen using the previous score cutoff value resulted in 57 hits, with 28 of them corresponding to approved drugs (Table 3 shows the top-scoring hits, their original indication, their score and predicted PPV value).

It should be noted that, whereas almost all the hits (with the exception of lacosamide, which is an already known anticonvulsant, and the benzodiazepine flurazepam) constitute valid options as starting points (novel scaffolds) to develop new AEDs though hit-to-lead and lead optimization strategies, not all of them are identically attractive as repurposing prospects, especially considering that epilepsy is a chronic condition that requires chronic medication. When considering a repurposing candidate, one should take into account its original indication (is it compatible with the intended new one?) and also the dose compatibility between the previous and the new indication (which depends on the effective concentrations required in each case, but also possibly on pharmacokinetic considerations: i.e. the drug levels reached in different organs may vary) [61, 62]. Systemic medications are more favorable as repurposing candidates for

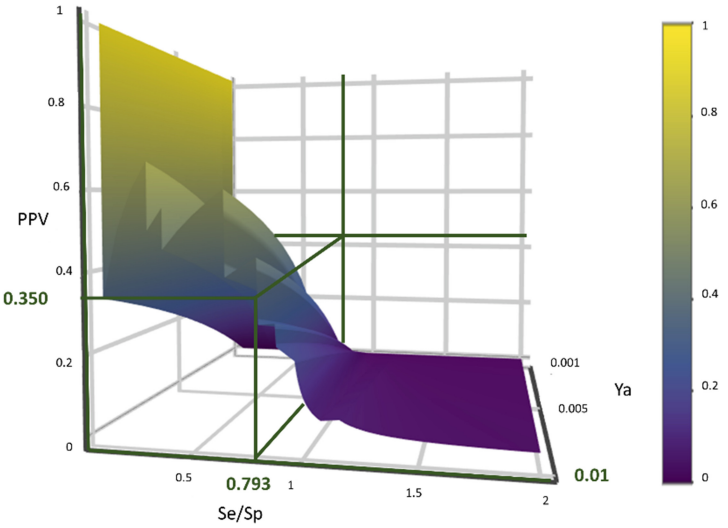
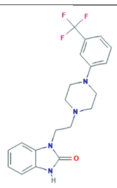
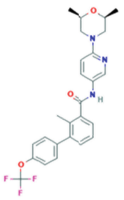


Fig. 4. PPV surface for the best 29-ensemble model.

systemic ailments. New therapeutic indications requiring equal or lower doses than the ones used for the original indication represent a more straightforward repurposing opportunity.

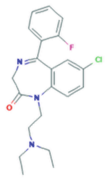
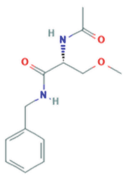
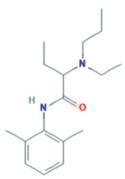
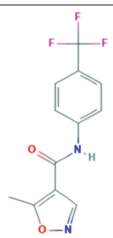
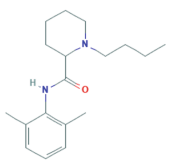
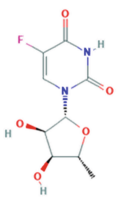
For instance, anticancer agents in Table 3 (sonidegib, doxifluridine) can be regarded as poor repurposing candidates due to their unfavorable safety profile (for instance, common side effects include muscle spasms, hair loss, fatigue, abdominal pain, nausea, headache, and weight loss).

Table 3. Top-scoring hits, their original indication, their score and predicted PPV value

Name	MIN Score	PPV% (Ya=0.01)	Structure	Original indication
Flibanserin	0.6185	33.43		Hypoactive Sexual Desire Disorder in Women
Sonidegib	0.5601	50.10		Antineoplastic Agent

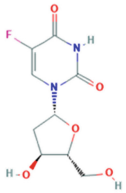
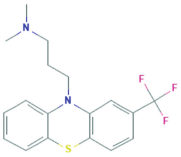
(continued)

Table 3. *(continued)*

Flurazepam	0.4952	55.66		Hypnotic
Lacosamide	0.4683	45.56		Anticonvulsant
Etidocaine	0.4537	50.10		Local anesthetic
Leflunomide	0.4142	47.48		Disease-modifying antirheumatic drug
Bupivacaine	0.3907	42.96		Local anesthetic or analgesia for surgery
Doxifluridine	0.3857	39.23		Treatment of stomach cancer

(continued)

Table 3. *(continued)*

Floxuridine	0.3788	39.24		Antineoplastic antimetabolite
Trifluprom azine	0.3783	39.23		Management of psychoses. Also to control nausea and vomiting

4 Conclusions

We have implemented a machine learning study to build ligand-based linear models capable of discriminating between active and inactive anticonvulsant drugs in the 6 Hz seizure test, one of the primary in vivo screens of ETSP. Using a random subspace strategy and Dragon conformation-independent models, we have obtained satisfactory individual classifiers, whose statistical behavior has though been improved by ensemble learning. The best performing ensemble was used in a prospective virtual screening experiment on DrugBank and Sweetlead databases, leading to 29 approved hits which are straightforward drug repurposing candidates. We will experimentally examine their activity in the 6 Hz model in the near future.

It should be highlighted that, whereas very valuable as a primary screen for AEDs with distinctive pharmacological profile, such as levetiracetam or brivaracetam, the 6 Hz models is still an acute seizure model with adequate throughput, but it is not a model of epilepsy, and it provides limited, insufficient evidence for a translational analysis. Animal models of seizure should be complemented with (more complex and low-throughput) animal models of epilepsy to grasp a better understanding of the perspective of a drug candidate as a treatment for human epilepsy, as suggested by the EPTS itself [6].

Virtual screening and computer-guided drug repurposing are excellent strategies to expedite the development of innovative medications, by exploiting previous knowledge on the pharmacological, toxicological and pharmacokinetic data of known drugs, and also by rescuing abandoned/shelved and discontinued drugs and drug candidates.

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