Chapter 15

Discovering New Antiepileptic Drugs Addressing the Transporter Hypothesis of Refractory Epilepsy: Structure-Based Approximations

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Abstract

Drug resistance represents a major obstacle to the success of epilepsy treatments, therefore intense investigations have been carried out to explain its origins. One of the most experimentally corroborated theories is the transporter hypothesis. It proposes that, at least for a subset of patients, the failure of the anticonvulsant drugs is caused by their inability to reach the molecular targets due to the regional overexpression or activation of efflux transporters. Among them, P-glycoprotein (P-gp) showed overactivity in drug-resistant patients as well as proved interactions with known anticonvulsant drugs. In this chapter we summarized the structure-based approximations employed to identify substrates/inhibitors of the glycoprotein, with special attention in describing the structural data available of the target- and the docking-based simulations. We also pointed out our results regarding the identification of new anticonvulsant candidates that avoid P-gp interactions by means of a sequential ligand-based and target-based screening, along with practical details related to this protocol.

Key words Refractory epilepsy, Docking, P-Glycoprotein, Virtual screening

1 The Transporter Hypothesis of Refractory Epilepsy

Despite the successful discovery of new antiepileptic drugs (AEDs) with better absorption, distribution, metabolism, excretion, and toxicity (ADME/TOX) profiles (or, in some examples, with novel mechanisms of action), the efficacy of drug treatment of epilepsy has not substantially improved over the years. According to the international consensus, refractory epilepsy is described as the failure to achieve seizure freedom with two or more well-tolerated AEDs, given in an appropriate manner [1]. About 30% of the patients fit to this definition, since they respond inadequately to known drug therapies. This fact represents a main concern for the experts in the field, who elaborated several hypotheses to explain the origins of the drug-resistant epilepsy.

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Abundant evidence acquired from epidemiological analysis, studies in animal models, and human epileptic tissues of patients undergoing surgical resection indicates that the refractory epilepsy is a multifactorial phenomenon. It might be associated with the development of tolerance during prolonged administration of the AEDs [2], the etiology and severity of the seizures [3], changes of the drug targets [3–6], alterations in neuronal connectivity [7, 8], blood–brain barrier dysfunctions [9], and genetic variants of the proteins involved in the biodistribution, metabolism, and mechanism of action of the drugs [10]. In addition to these causes, the *transporter hypothesis* claims that the failure of AEDs in some refractory patients is originated by their inability to reach the molecular targets, due to the overexpression or activation of efflux transporters in brain tissues [11]. This mechanism of drug resistance is highly supported by experimental data [11–25] and provides a possible explanation about one important characteristic of refractory epilepsy: patients that fail to control the seizures with one AED have a small chance (lower than 10%) to control them by other AEDs, even with drugs that act through the interaction with different molecular targets [10, 11, 26].

The most-studied efflux transporter is P-glycoprotein (P-gp). It is a member of the ATP-binding cassette (ABC) transporter family, and it is expressed in brain cells (neural cells, glial cells, and capillary endothelium at the blood–brain barrier) as well as in other barriers and excretory tissues, with the function of detoxifying them by preventing exogenous compounds from entering susceptible organs [27, 28]. In contrast to this beneficial effect, the regional overactivity of P-gp in drug-resistant patients limits the access of AEDs into the brain targets, rendering them ineffective [13, 21, 22, 29, 30]. Moreover, studies about the interaction between P-gp and AEDs confirmed that phenytoin, phenobarbital, topiramate, lamotrigine, levetiracetam, oxcarbazepine, and eslicarbazepine acetate (and some of their metabolites) are substrates or inhibitors of the P-gp [31–35]. In this context, it became advisable to include in the early phases of AED discovery the analysis of the interactions of the newly designed anticonvulsants with P-gp.

According to the characteristics of the binding, compounds can be categorized as substrates, inhibitors, or modulators of P-gp. Substrates are actively transported by P-gp, whereas inhibitors affect the transporting function. Modulators interact with active sites distinct from the substrates, reducing the strength of its interactions by a negative allosteric binding [36]. Since both inhibitors and modulators impair the transport function, they are frequently named equally as inhibitors [37]. Additionally, as modulators, inhibitors, and substrates interact with P-gp in one way or another, here we will refer them together as *binders*.

There are several biological assays developed to test the ability of compounds to interact with P-gp [38, 39]. Among them, in vitro transport experiments are recommended by the US Food and Drug Administration as the preferred data to decide if a drug

is a P-gp binder. They suggested a bidirectional transport assay using cultured cells as the initial test, followed by the validation that the efflux is inhibited by the presence of one or more inhibitors [40]. The in vitro methods with Caco-2 cells are the most frequently employed, followed by the MDCKII-MDR1 cells (multidrug resistance protein-1-transfected Madin–Darby canine kidney cells) [41]. However, any experimental screening to evaluate P-gp interactions with new active compounds is expensive and time demanding, so computational models provide a valuable and complementary tool for the virtual recognition of P-gp binders.

2 In Silico Approaches to Predict P-gp Binders

The impact of P-gp on the drug resistance to treat not only epilepsy but also other diseases (such as cancer, Alzheimer, and HIV [42–45]) has promoted the development of in silico studies to identify P-gp binders. It is known that P-gp has the capability to interact with unrelated structures, and this poli-specificity makes it difficult to find a common pattern for the recognition of binders. However, numerous ligand-based approaches were developed to find the requirements shared by small molecules to be P-gp substrates or inhibitors. The initial pharmacophoric patterns proposed the importance of aromatic rings and lipophilic centers, basic nitrogen atoms, and hydrogen-bonding interactions [46–48]. After that, other ligand-based approaches were developed to predict P-gp binders, which include new pharmacophoric patterns, machine-learning algorithms, and quantitative structure–activity relationship (QSAR) studies among others [47, 49–60]. Details of the ligand-based models are widely explained in a previous chapter of this book. Some of them present a high prediction accuracy for given classes of drugs or drug candidates, with the additional advantage of being less computationally demanding than target-based approaches.

Structure-based methods deal with the high computational cost (particularly for virtual screening campaigns with large datasets), but they are able to provide atomic details on the protein–ligand interactions. Therefore, these approaches allow the prediction of the binding modes between new (or known) compounds and the biological targets as well as the structural optimization of the ligands (to improve/avoid their interactions with the macromolecules). Of course, the application of structure-based approximations implies the knowledge of the target at atomic level.

2.1 Tridimensional Structure of P-gp

The elucidation of the 3D structure of membrane proteins from crystallography still represents a challenge in structural biology. Regarding crystals of transporters in complex with some substrates, the experiments get even more problematical because the low binding affinities increase the difficulty to solve their positions.

In 2009 Aller and coworkers elucidated the first X-ray structure of the eukaryotic (*Mus musculus*) P-gp, improving enormously the knowledge of the protein architecture at atomic level [61]. Before this finding, the 3D structure of P-gp was modeled by comparative analysis (homology-modeling techniques) with templates that share low sequence identity to the human protein, such as the bacterial ABC transporter MsbA [62–64]. Conversely, the sequence of mouse P-gp has more than 80% sequence identity to human P-gp and similar size [65], suggesting a high level of conservation of the 3D structure.

Additionally, Aller and coworkers crystallized two protein–ligand complexes providing the first structural details about how P-gp interacts with their binders [61]. The reported apo and drug-bound structures showed inward-facing conformations of P-gp, which correspond to the initial stage of the transport cycle. Subsequently, other experimental models were also elucidated and deposited in the protein data bank [66] improving the information about the overall 3D structure of P-gp and the architecture of the binding sites (Table 1).

As other ABC proteins, P-gp employs the hydrolysis of ATP to efflux out of the cell substrates across the biological membrane. It comprises two pseudosymmetric halves. Each one has six transmembrane helices (TMs) connected with loops and short helices to one cytosolic nucleotide-binding domain (NBD) (Fig. 1).

Table 1

Experimental 3D models of P-gp deposited in the protein data bank after the mouse structures elucidated by Aller in 2009 [61]

Date	Authors	Related PDB codes (resolution)	Organism
2009	Aller et al. [61]	3G5U (3.80 Å)	*Mus musculus*
		3G60 (4.40 Å)	
		3G61 (4.35 Å)	
2012	Jin et al. [67]	4F4C (3.40 Å)	*Caenorhabditis elegans*
2013	Li et al. [68]	4M1M (3.80 Å)	*Mus musculus*
		4M2S (4.40 Å)	
		4M2T (4.35 Å)	
2013	Ward et al. [69]	4KSB (3.80 Å)	*Mus musculus*
		4KSC (4.00 Å)	
		4KSD (4.10 Å)	
2015	Szewczyk et al. [70]	4Q9H (3.40 Å)	*Mus musculus*
		4Q9I (3.78 Å)	
		4Q9J (3.60 Å)	
		4Q9K (3.80 Å)	
		4Q9L (3.80 Å)	

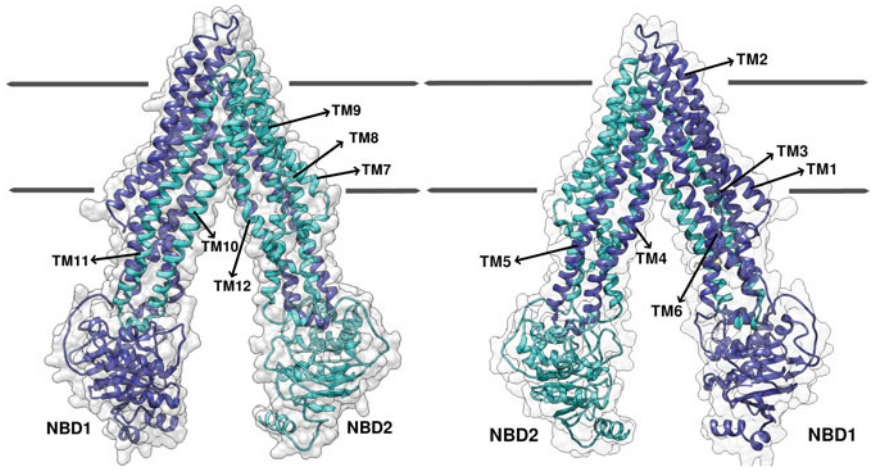

Fig. 1 Overview of the crystal structure of mouse P-gp (PDB code 4Q9H). Transmembrane domains are labeled (TM). *Horizontal lines* approximate the region of the lipid bilayer

Regarding the binding sites for substrates and inhibitors, previous analyses have proposed up to seven binding regions located into the internal cavity of the protein, which is generated by the two sets of TMs (TMs 1–3,6,10,11 and TMs 4,5,7–9,12) [71, 72]. The existence of multiple binding sites (some of them overlapped) in the P-gp internal cavity is consistent with the poli-specificity observed for the protein. Moreover, this cavity has a volume around 6000 Å^3, which is big enough to accommodate more than one binder simultaneously in different subsites [61] (Fig. 2). Recently, another potential binding site has been detected by X-ray crystallography [70]. It is placed on the exterior of the P-gp structure but close to the region proposed as a potential intramembranous access of the substrates to the cavity [73, 74]. The authors suggested that there is an initial weak interaction of the substrates with this site before achieving their final position into the internal cavity [70] (Fig. 2).

2.2 In Silico Predictions of P-gp Binders Through Target-Based Methods

The structural information of P-gp provides the starting point for the application of target-based methods of drug design. Among them, molecular docking protocols were employed to locate P-gp putative binding sites and to predict the binding affinities of small molecules. Note that the results of a docking protocol are highly dependent on the experimental data available, the predefined scoring functions, and the conformational search algorithms provided by the software. Consistently, it is always convenient to test a diversity of docking alternatives and appropriately validate the suitability of the protocol to reproduce the experimental conformation of ligand–target complexes, if they are available. In the same line, it is opportune to test their ability to discriminate known binders from non-binders through their docking score. It is expected that they predict better affinities (i.e., lower calculated binding energies) for binders.

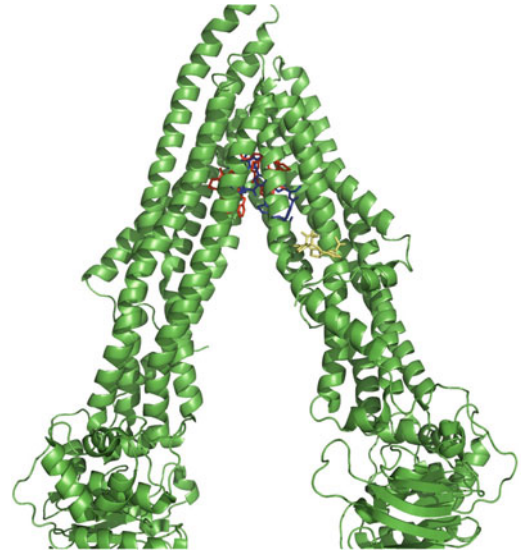

Fig. 2 Overview of P-gp co-crystallized with valine–cyclopeptide structure (PDB code 4Q9J). Two molecules of the ligand are located in the internal cavity (highlighted in *blue*), whereas another is placed on the exterior of the structure (highlighted in *yellow*). The position of the other two ligands (phenylalanine cyclopeptide, co-crystallized in the PDB complex 4Q9L) was also included in *red*, to show the versatility of the active site

Becker and collaborators constructed a P-gp homology model based on the 3D structures of the transporters Sav1866 and MsbA to dock the ligands verapamil, rhodamine B, colchicine, and vinblastine into the binding cavity [64]. The results showed good correlation with the experimental data available since the docking poses interact with residues previously identified in the active site. Pajeva et al. employed the structure of mouse P-gp (PDB code: 3G61) as template to construct a P-gp human 3D model, and they docked a small set of quinazolinone inhibitors to support the results obtained from pharmacophoric patterns about the common structural features of P-gp binders [75]. Later on, other authors evaluated the ability of docking programs to discriminate known binders from non-binders [49, 60, 76, 77]. They constructed bigger test sets than Becker and Pajeva with different chemical backbones to consider the promiscuity of the P-gp binding sites. The final proposition was to employ docking algorithms to predict the P-gp binding affinities of untested molecules.

Chen et al. evaluated the prediction capability of molecular docking by using the two drug-bound P-gp structures provided by Aller et al. (PDB codes: 3G60 and 3G61, Table 1). They docked 157 substrates and 88 non-substrates with Glide docking software (http://www.schrodinger.com/Glide) and scored them by the two Glide modes: SP (standard precision) and XP (extra

precision). The authors concluded that the docking protocols were unable to clearly discriminate substrates from no substrates by using the best score criterion [49].

Dolghih and coworkers applied docking protocols to analyze their capacity to differentiate 30 binders (26 drugs and four metabolites) from 98 presumed non-binders (metabolites). They employed the target structure provided by Aller (PDB code: 3G60) and run rigid and flexible docking (with induced fit algorithms). The force fields were provided by Glide SP, Glide XP, and MM-GB/SA scoring functions [76]. Additionally, they tested 13 binders and 34 non-binders selected from a dataset of FDA-approved drugs. The active compounds of the set were positive in two experimental assays (monolayer efflux and calcein-AM inhibition assays) whereas the non-binders were negative for both experiments. Finally they benchmarked the docking conditions with a blind test on a series of peptidic cysteine protease inhibitors. The authors concluded that flexible receptor models have the ability to differentiate known binders from non-binders. They proposed that the better results in flexible docking might be associated with the mobility of the binding site residues but also with the low resolution of the target structure. They also suggested that P-gp substrates could bind deeper in the P-gp cavity than the ligands in the crystallized complex [76].

Dolghih et al. also docked rhodamine B, a known P-gp substrate with experimental binding data available [78, 79], to validate if docking can predict accurately the geometry of experimental complexes. In the same line, they strip and docked the ligand (a cyclic peptide) back into the crystal structure 3G60 (named QZ59-RRR). Again, the flexible receptor poses reproduced better the experimental binding interactions. Interestingly, one of the best docking conditions found in this investigation was then applied by the authors to estimate the efflux ratio of the molecules (the ratio of their two transport rates in opposite directions: basal to apical and apical to basal across a single layer of cells) as a measure of their brain penetration [80].

On the other hand, Bikardi et al. combined ligand-based and target-based methods to find a cost-effective protocol to predict potential P-gp substrates and their molecular interactions [50]. The identification of possible P-gp substrates was in charge of a support vector machine method (with a training dataset of 197 known P-gp substrates and non-substrates), and the docking calculations were employed only to predict the interactions at atomic level of the selected compounds. The authors validated the docking protocol (Autodock Vina software) by re-docking the QZ59RRR ligand to the mouse X-ray P-gp structure. They found an acceptable agreement between the experimental and predicted ligand conformations (RMSD value of 1.27). However, the binding predictions were performed on a human P-gp model as the target. The authors constructed a 3D homology model (using mouse P-gp as template) and tested it by docking rhodamine B.

The results of this docking calculation were consistent with experimental data [78, 79]. The algorithms are available in a Web server, which enables the users to predict if a compound is a P-gp substrate, as well as its binding conformation into the P-gp active sites.

Bikardi et al. did not use docking for classifying P-gp binders from non-binders. Conversely, Klepsch and collaborators compiled a large set of 1076 inhibitors and 532 non-inhibitors to test the capacity of the scoring functions implemented in GOLD package to differentiate them [60]. They employed a model of the human P-gp as target, which was constructed from murine P-gp (PDB code 3G5U). Initially, the ligands were docked in two protonation states with ChemScore or GoldScore functions. Then, the resulting docking poses were rescored with five scoring functions: ChemScore, GoldScore, Astex Statistical Potential (ASP), Piecewise Linear Potential (ChemPLP), and XScore [60]. In summary, 20 final models were obtained and their prediction capabilities were investigated. The best one was based on ChemScore, and it was able to predict 76% of P-gp inhibitors and 73% of non-inhibitors. Additionally, the authors combined the results obtained from ChemScore-based docking with the log P values of the compounds, which relates to the ability of the compounds to cross the membranes by diffusion. It caused a slight improvement in the prediction of true inhibitors, with values in the confusion matrix of 0.81 for sensitivity (i.e., 81% of the inhibitors predicted), at expenses of a decrease in the detection of non-inhibitors (0.69 of specificity) [60]. Note that this approach is philosophically consistent with the enhanced sensitivity of the CETA assay [35] to identify lipophilic weak substrates of P-gp by removing the diffusion component from the permeability assay.

It is worth mentioning that docking protocols are unable to provide a complete explanation about the ligand interactions during efflux cycle in all the models mentioned before. More than a few doubts remained unsolved about the binding sites' specific locations for substrates and/or inhibitors. Moreover, during the course of the efflux cycle, P-gp undergoes large-scale conformational changes to pump the drugs out of the cellular membranes, and the mechanisms of these conformational transitions are still unclear. In that direction, numerous attempts have been performed by means of molecular dynamic simulations to better understand the mechanisms of drug uptake and binding in a flexible protein like P-gp [81–89]. The studies confirm the significant conformational change that facilitates the extrusion of the molecules and the importance of the TM12 and TM6 regions for the flexibility of the macrostructure.

2.3 Structure-Based Design of New Anticonvulsants to Treat Refractory Epilepsy

The early recognition of potent anticonvulsant candidates with no interaction with P-gp represents a useful strategy to design anticonvulsant drugs. On the other hand, the virtual identification of compounds with strong interactions with P-gp could be implemented for developing inhibitors for adjuvant therapies.

This last strategy has serious limitations since it implies to cancel the physiological role of the glycoprotein (a first line of defense in several barriers), and it involves the development of very selective and specific inhibitors to minimize side effects.

To the extent to our knowledge, the docking-based protocols mentioned before in this chapter have not been employed to find new anticonvulsants that avoid interactions with P-gp. On the contrary, we and other authors have run a virtual screening campaign to identify anticonvulsants for the treatment of P-gp-mediated drug-resistant epilepsy [77]. Initially we applied four ligand-based models on the ZINC 5 [90] and DrugBank [91–93] databases to propose new possible non-substrates of P-gp with anticonvulsant properties. Briefly, a topological discriminant function was employed to identify active compounds against the maximal electroshock-induced seizure (MES) test, and a three-model ensemble of 2D classifiers was used to differentiate P-gp substrates from non-substrates.

From the ligand-based screening, 380 candidates were selected for a second round of analysis by docking simulations. We first analyze the capacity of different docking software and conditions to discriminate known binders from non-binders of the test set, which was constructed after a thorough analysis of the bibliographic data. Special attention was given to the selection of the compounds of the test set, since there is some controversy in the literature regarding the classification of substrates/inhibitors and non-substrates/non-inhibitors. For example, Polli et al. classifies verapamil as non-substrate according to the monolayer efflux experiment in MDCK cells [94], whereas Feng et al. consider it as substrate in the same assay [95]. Doan et al. [96] reports fluoxetin as a non-substrate (monolayer efflux experiment in MDCKII-MDR1 cells), but in the assay of calcein-AM (CAM) inhibition (same cellular line), it behaves as an inhibitor [97]. In order to construct a representative and diverse set, the biological results from multiple publications were considered and priority was given to in vitro assays over in vivo tests [65]. After a thorough analysis, we included in the test set those substrates/inhibitors that were detected in two or more publications in different assays (if possible more than three). Conversely, we considered non-binders those compounds found as non-substrates preferably in two or more different assays and not reported as substrate/inhibitors (or reported in only one test). The compounds reported with some controversy in the results (e.g., one test where the compound was considered substrate/inhibitor and another where it behave as non-substrate) were discarded.

We benchmarked Glide, Autodock, and Autodock Vina software [65]. The compounds were docked into a homology model of human P-gp based on the mouse P-gp structure as template (PDB code: 3G61). The target was considered either rigid or flexible. Two different sets of amino acids were allowed to move in the

flexible simulations. In one model (model A) we allowed the movement of the amino acids that interact with the experimental ligands in the mouse complexes (Phe-335, Phe-343, Phe-728, Phe-732; PDB codes 3G60 and 3G61). For model B, we examined the conformation of the flexible residues in model A after the docking simulations. We found that Phe-343 and Phe-978 showed different conformations depending on the ligand, whereas Phe335, Phe732, and Phe728 adopted practically the same conformation in all the tested compounds. Therefore we choose another set of flexible residues that includes Phe-343, Phe-978, and other amino acids (Tyr-307, Tyr-953) that interact with the compounds of the test set according to the docking results with model A. The results were analyzed by means of the receiver-operating characteristic (ROC) curves for each docking system [98] to decide the most favorable docking conditions and to choose the limiting docking score (best threshold value) that can be used to discern between binders and non-binders classes.

The ROC curves graph the relation between the false positive rate (on the X axis) and the true positive rate (on the Y axis) for all possible threshold levels (see as example Fig. 3). These two values are defined as follows:

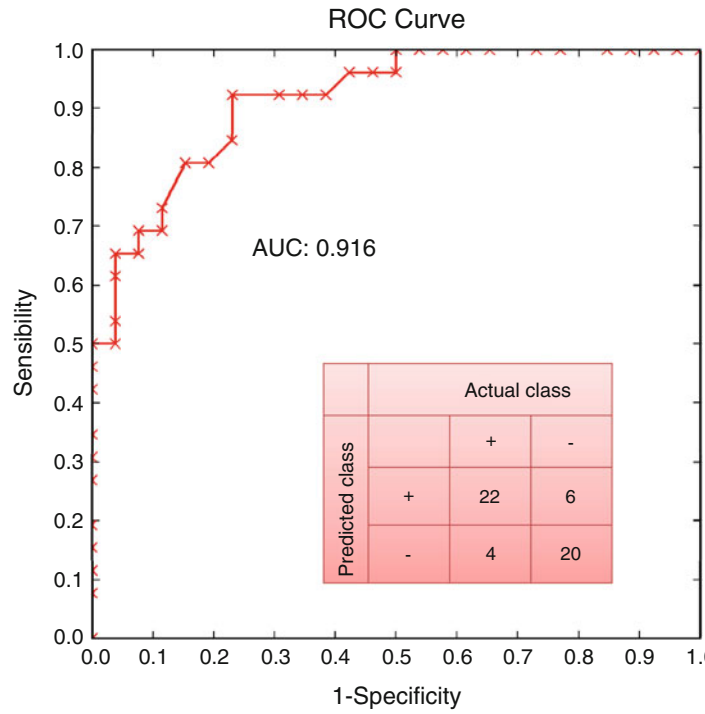

Fig. 3 ROC-type curves obtained for the best simulation. The confusion matrix with threshold −7.4 shows the number of well-categorized binders (22 of 26) and non-binders (20 of 26)

False positive rate = FP/(TN + FP)

True positive rate = TP/(TP + FN)

where TP means true positives, FN false negatives, FP false positives, and TN true negatives. The true positive rate is also defined as sensitivity and the false positive rate is also named as 1-specificity, since specificity is the rate of true negatives: TN/(TN + FP).

The ROC plots provide an objective picture of the methods' performance through the area under the curve (AUC) [98]. A random classification would give an AUC of 0.5, whereas a perfect classification would give an AUC of 1. Therefore, the AUC of the "real" models ranges between these two values. The analyses of the curves are useful to compare different methods and to have an overview of their capacity to discriminate known active from known inactive compounds.

As mentioned before, to construct the ROC curve we need to determine the selectivity and specificity values. Continuous data (as docking scores) requires a threshold value that divides the predicted active and inactive compounds. Depending on this cutoff, the selectivity and specificity will vary, and each pair of values represents a point in the curve.

On the other hand, for virtual screening purposes, we need to select the best threshold value that allows deciding which new compounds are good candidates to experimental analysis. If this cutoff is selected so that the sensitivity of the model increases (high rate of true positives), the specificity will decrease and we will expect a high rate of false positive compounds. On the contrary, if specificity is favored over sensitivity, more true positives will be classified as inactive.

There are several criterions to balance both sensitivity and specificity, and the threshold values are selected according the given system. For example, the highest G-mean value (square root of the product between selectivity and specificity) could be used to select the best cutoff for virtual screening. Another practical criterion is based on the accessibility of the experimental assays. As the resources involved in the experimental test are limited, it would be useful to minimize the evaluation of false positive candidates. This criterion prioritizes specificity over sensitivity, at expenses of losing active compounds.

In our investigation the best ROC curve was achieved by means of Autodock Vina with flexible receptor (model B) and ligands at physiological pH. The resulting simulation is shown in Fig. 3, with an area under the ROC curve of 0.916 (best threshold of docking score: −7.4). This protocol is able to predict the 85 % of the binders (sensitivity value of 0.85) and the 77 % of non-binders (specificity value of 0.77) with a global accuracy value of 0.81 [65].

Finally we performed the docking simulation to the 380 compounds selected by the ligand-based virtual screening. According to the ROC curves, we considered as P-gp binders the structures that showed a docking score lower than −7.4 kcal/mol. With this

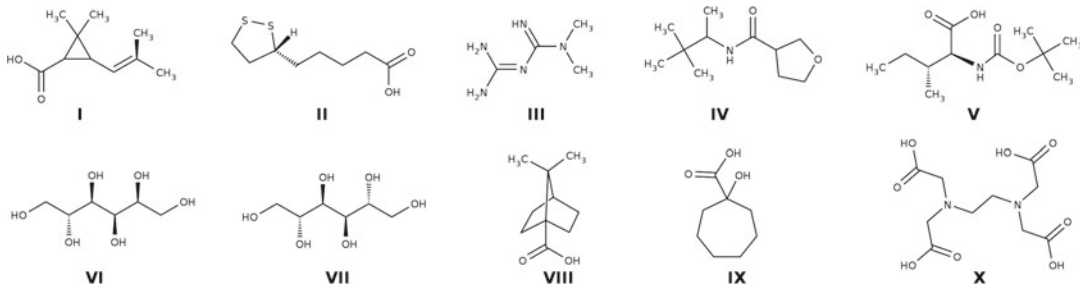

Fig. 4 Compounds selected by sequential virtual screening that showed anticonvulsant activity against MES test. I, chrisantemic acid; II, 7,7-dimethyl-1-norbornane carboxylic acid; III, thioctic acid; IV, metformin; V, mannitol; VI, sorbitol; VII, *N*-(*tert*-butoxycarbonyl)-l-isoleucine; VIII, 1-hydroxycycloheptanecarboxylic acid; IX, *N*-(3,3-dimethylbutane-2-yl)-2-methylfuran-3-carboxamide; X, EDTA [65]

threshold, 275 structures passed the docking filter as non-binders, evidencing a high level of consensus between the ligand-based and target-based protocols (more than 72 % of the initial structures were recovered by docking). From them, ten diverse molecules were selected for acquisition and subsequent pharmacological evaluation (Fig. 4). All showed anticonvulsant properties in animal models of acute seizures (MES test), proving the accuracy of our predictions in relation to the protective effects of the candidates [77].

3 Conclusions

P-glycoprotein is probably one of the most studied antitargets (together with hERG channel and CYP3A4 enzyme) due to its participation in drug resistance and drug interactions.

Particularly, the P-gp awareness for the design of new anticonvulsants represents an interesting strategy to treat P-gp-mediated refractory epilepsy. In this context, target-based methods offer a virtual alternative to ligand-based protocols, since they might discern possible binders from non-binders using, for example, the values of the docking scores.

Docking on P-gp deals with the intrinsic limitations of the software (characterized by specific-search algorithms and force fields) to correctly quantify the binding energies of the complexes. Additionally, human P-gp is a very flexible membrane protein and its experimental structure is not currently available. Therefore, the simulations have to be run on experimental structures of mouse P-gp (which show low resolution in the position of the amino acid side chains) or by comparative models of the human target. Despite these difficulties, the docking protocols mentioned in this chapter showed, in general, an acceptable performance. Additionally they were able to reproduce the binding modes of known binders and could provide atomic details on the new protein–ligand complexes.

The final step in the virtual screening campaigns is to test the candidates in adequate experimental models. The implementation of models of drug-resistant epilepsy, taking into consideration overexpression of P-gp, would optimize the process of selection of compounds. They could serve to identify new chemical entities that control resistant patients and to better understand the mechanisms of drug resistance.

In vitro cell assays include for example the use of isolated brain capillaries, primary or immortalized brain endothelial cells, and immortalized cells from peripheral tissues (some of them mentioned before in this chapter) [99, 100]. On the other hand, in vivo models of drug-resistant seizures associated with overexpression of P-gp, such as the model of seizure induced by 3-mercaptopropionic acid, are available [100].

We believe that the sequential screening of large databases with ligand-based filters coupled by target-based protocols represents an opportunity for the discovery of new anticonvulsants that evade P-gp interactions in a timely and cost-efficient manner. Moreover, the prediction of the P-gp-binding modes might serve to optimize the new leaders without losing their anticonvulsant action. These investigations, in conjunction with suitable biological models, could afford new effective drugs in patients with P-gp-mediated refractory epilepsy.

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