A Machine Learning-Based Prediction Platform for P-Glycoprotein Modulators and Its Validation by Molecular Docking

Onat Kadioglu and Thomas Efferth *

Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, 55128 Mainz, Germany; kadioglu@uni-mainz.de

* Correspondence: efferth@uni-mainz.de; Tel.: +49-6131-3925751; Fax: +49-6131-3923752

Received: 9 September 2019; Accepted: 20 October 2019; Published: 21 October 2019

Abstract: P-glycoprotein (P-gp) is an important determinant of multidrug resistance (MDR) because its overexpression is associated with increased efflux of various established chemotherapy drugs in many clinically resistant and refractory tumors. This leads to insufficient therapeutic targeting of tumor populations, representing a major drawback of cancer chemotherapy. Therefore, P-gp is a target for pharmacological inhibitors to overcome MDR. In the present study, we utilized machine learning strategies to establish a model for P-gp modulators to predict whether a given compound would behave as substrate or inhibitor of P-gp. Random forest feature selection algorithm-based leave-one-out random sampling was used. Testing the model with an external validation set revealed high performance scores. A P-gp modulator list of compounds from the ChEMBL database was used to test the performance, and predictions from both substrate and inhibitor classes were selected for the last step of validation with molecular docking. Predicted substrates revealed similar docking poses than that of doxorubicin, and predicted inhibitors revealed similar docking poses than that of the known P-gp inhibitor elacridar, implying the validity of the predictions. We conclude that the machine-learning approach introduced in this investigation may serve as a tool for the rapid detection of P-gp substrates and inhibitors in large chemical libraries.

Keywords: artificial intelligence; drug discovery; machine learning; molecular docking; multidrug resistance; P-glycoprotein

1. Introduction

ATP-binding cassette (ABC) transporters are energy-dependent efflux pumps responsible for the active efflux of drugs, thereby reducing their intracellular concentration. Due to overexpression of ABC transporters in tumor cells, multidrug resistance (MDR) develops, which leads to the failure of chemotherapy with fatal consequences for cancer patients [1]. P-glycoprotein, being a well-known member among the ABC transporter family, is encoded by the ABCB1/MDR1 gene. It is an important determinant of MDR [2–4] and upregulated in many clinically resistant and refractory tumors [5,6]. Its overexpression in tumor cells is associated with efficient extrusion of a large number of established anticancer drugs and natural cytotoxic products out of cancer cells, representing a major drawback of cancer chemotherapy [7]. Resistance is either inherently present or will be acquired during chemotherapy [8–10]. Hence, P-glycoprotein (P-gp) represents an important target to search for pharmacological inhibitors to overcome MDR [11]. Targeting P-gp to overcome MDR is of importance to achieve higher success rates for chemotherapy. The concept is to combine P-gp inhibitors with established chemotherapy drugs to resensitize tumors [12–15].

Machine learning and artificial intelligence are recently acquiring increasing interest in the area of drug discovery [16–18] because these methods have an enormous potential to speed up the preclinical
development processes at minimal costs. For this purpose, we utilized a machine learning strategy in order to establish a prediction platform that allows to predict whether a given compound behaves as a substrate or an inhibitor of P-gp.

Available natural compound databases serve as an invaluable source to identify novel lead compounds that possess activity against certain diseases or disorders by focusing on particular target biomarker proteins. As a majority of established anticancer drugs are of natural origin [19], natural products may serve as lead compounds for derivatization to obtain novel chemical entities with improved pharmacological features. Analyses of the interaction between the compounds and the target protein with molecular docking provide clues about the possible binding mode and binding energy, as we reported before [11,20,21]. Selecting P-gp as target protein, the interaction of test compounds can be compared with that of known P-gp inhibitors, such as verapamil, valspodar, tariquidar, or elacridar, in order to assess their binding properties, docking poses, and binding energies. In those cases, where the test compounds yielded by using the P-gp modulator prediction platform possess similar docking poses and comparable binding energies as known inhibitors, it could be concluded that these compounds may be potential P-gp inhibitors.

In the present study, we used machine learning strategies to establish such a P-gp modulator prediction platform for compounds by using defined chemical descriptors to predict whether a given compound can behave as a substrate or an inhibitor of P-gp. Selected compounds from inhibitor or substrate classes were subjected to molecular docking for further verification and compared with known P-gp inhibitors and substrates.

2. Material and Methods

2.1. Preparation of Compound List and Calculation of Chemical Descriptors

For the P-gp modulator/non-modulator prediction model, a compound list with modulators and non-modulators from Broccatelli et al. [22] was used. Compounds for learning and validation steps were randomly selected. Thirty-two modulator and thirty-two non-modulator compounds were used for the learning step, while 16 modulator and 16 non-modulator substances were used for the validation step (Table 1). For the P-gp inhibitor/substrate prediction model, a list of P-gp substrates and inhibitors was prepared by referring to the literature [23], yielding a total of 60 compounds (34 inhibitors, 26 substrates). Again, compounds for learning and validation steps were randomly selected. Forty compounds (20 inhibitors, 20 substrates) were used for learning and model establishment. The remaining 20 compounds (14 inhibitors, 6 substrates) were used for the external validation step (Table 2).

| Table 1. Compounds selected for learning and external validation for the P-glycoprotein (P-gp) modulator/non-modulator prediction model. |
|---|---|---|---|
| **Learning Set** | **External Validation Set** |
| **Compound** | **Category** | **Compound** | **Category** | **Compound** | **Category** |
| Escitalopram | Modulator | Hydroxyzine | Non-modulator | Terfenadine | Modulator |
| Simvastatin acid | Modulator | Oxybutynin | Non-modulator | Prazosin | Modulator |
| Neostigmine | Modulator | Ethosuximide | Non-modulator | Prednisone | Modulator |
| Zolmitriptan | Modulator | Warfarin | Non-modulator | Chloroquine | Modulator |
| Atomoxetine | Modulator | Mexilitene | Non-modulator | Lopinavir | Modulator |
| Methysergide | Modulator | Sulpiride | Non-modulator | Prednisolone | Modulator |
| Famiciclovir | Modulator | Thiopental | Non-modulator | Vincristine | Modulator |
| Lovastatin acid | Modulator | Lamotrigine | Non-modulator | Sertraline | Modulator |
| Darifenacin | Modulator | Diphenhydramine | Non-modulator | Loperamide | Modulator |
| Paliperidone | Modulator | Enoxacin | Non-modulator | Etoposide | Modulator |
| Tropicamid | Modulator | Methylenidate | Non-modulator | Indinavir | Modulator |
| Aprepitant | Modulator | Itraconazole | Non-modulator | Dipyridamole | Modulator |
| Apomorphine | Modulator | Nortriptyline | Non-modulator | Mitoxantrone | Modulator |
| Compound                  | Category | Compound         | Category | Compound                  | Category | Compound         | Category |
|---------------------------|----------|------------------|----------|---------------------------|----------|------------------|----------|
| Cetirizine                | Modulator| Galantamine      | Non-modulator | Cimetidine                | Modulator|                  |          |
| Cyclosporin A             | Modulator| Ramelteon        | Non-modulator | Bromocriptine             | Modulator|                  |          |
| Labetalol                 | Modulator| Rivastigmine     | Non-modulator | Reserpin                  | Modulator|                  |          |
| Amisulpride               | Modulator| Ropivacaine      | Non-modulator | Oxprenol                  | Non-modulator|                  |          |
| 5-Hydroxymethyl           | Modulator| Zonisamide       | Non-modulator | Alprazolam                | Non-modulator|                  |          |
| tolterodine               |          |                  |          |                           |          |                  |          |
| Cabergoline               | Modulator| Zolpidem         | Non-modulator | Oxcarbazepine             | Non-modulator|                  |          |
| Ximelagatran              | Modulator| Sulfasalazine    | Non-modulator | Tolterodine               | Non-modulator|                  |          |
| Hoechst 33342            | Modulator| Metoclopramide   | Non-modulator | Zaleplon                  | Non-modulator|                  |          |
| Rhodamine 123            | Modulator| Nalmefene        | Non-modulator | Cyclobenzaprine           | Non-modulator|                  |          |
| Actinomycin D             | Modulator| Oxycodone        | Non-modulator | Nimodipine                | Non-modulator|                  |          |
| Olanzapine                | Modulator| Topiramate       | Non-modulator | Riluzole                  | Non-modulator|                  |          |
| Ramilidine                | Modulator| Hydrocodone      | Non-modulator | Tiagabine                 | Non-modulator|                  |          |
| Astemizole                | Modulator| Rosuvastatin     | Non-modulator | Nalluphine                | Non-modulator|                  |          |
| Verapamil                 | Modulator| Tropisetron      | Non-modulator | Duloxetine                | Non-modulator|                  |          |
| Ziprasidone               | Modulator| Varenicline      | Non-modulator | Pravastatin acid          | Non-modulator|                  |          |
| Chlorpromazine            | Modulator| Clemastine       | Non-modulator | Promazine                 | Non-modulator|                  |          |
| Clozapine                 | Modulator| Clonazepam       | Non-modulator | Bromazepam                | Non-modulator|                  |          |
| Trimepothromid            | Modulator| Ropinirole       | Non-modulator | Lorazepam                 | Non-modulator|                  |          |
| Paroxetine                | Modulator| Solifenacin      | Non-modulator | Mirtazapine               | Non-modulator|                  |          |

Table 2. Compounds selected for learning and external validation for the P-gp inhibitor/substrate prediction model.

| Compound          | Category | Compound | Category | Compound          | Category | Compound | Category |
|-------------------|----------|----------|----------|-------------------|----------|----------|----------|
| Ginsenoside       | Inhibitor| Epirubicin| Substrate | Agosterol         | Inhibitor| Colchicin| Substrate |
| Laniquidar        | Inhibitor| Etoposide| Substrate | Amiodarone        | Inhibitor| Dexamethazone| Substrate |
| Loratidine        | Inhibitor| Fexofenadine| Substrate | Amorin            | Inhibitor| Digoxin| Substrate |
| Mibefradil        | Inhibitor| Hoechst 33342| Substrate | Apigenin          | Inhibitor| Docetaxel| Substrate |
| Naringenin        | Inhibitor| Idarubicin| Substrate | Atorvastatin      | Inhibitor| Doxorubicin| Substrate |
| Pgp-4008          | Inhibitor| Irinotecan| Substrate | Atovaquone        | Inhibitor| Daunorubicin| Substrate |
| Phloretin         | Inhibitor| Kaempferol| Substrate | Biochanin         | Inhibitor|          |          |
| Quercetin         | Inhibitor| Loperamide| Substrate | Biricodar         | Inhibitor|          |          |
| Quinine           | Inhibitor| Mitomycin| Substrate | Catechin          | Inhibitor|          |          |
| Rotenone          | Inhibitor| Mitoxantrone| Substrate | Celoperazine      | Inhibitor|          |          |
| Sakuranetin       | Inhibitor| Ondansetron| Substrate | Chrysine          | Inhibitor|          |          |
| Sertraline        | Inhibitor| Paclitaxel| Substrate | Cyclosporine      | Inhibitor|          |          |
| Sinensetin        | Inhibitor| Procyanidin B2| Substrate | Diltiazem         | Inhibitor|          |          |
| Stigmasterol      | Inhibitor| Rhodamine 123| Substrate | Elacridar         | Inhibitor|          |          |
| Syringaresinol    | Inhibitor| Tenoposide| Substrate |          |          |          |          |
| Tamoxifen         | Inhibitor| Topotecan| Substrate |          |          |          |          |
| Tariquidar        | Inhibitor| Vinblastine| Substrate |          |          |          |          |
| Valspodar         | Inhibitor| Vincristine| Substrate |          |          |          |          |
| Verapamil         | Inhibitor| Vindesine| Substrate |          |          |          |          |
| Zosuquidar        | Inhibitor| Vinorelbine| Substrate |          |          |          |          |
Data Warrior software is a multipurpose chemistry data visualization and data analysis program that calculates various molecular descriptors and properties for a given set of compounds. It was used to calculate the chemical descriptors as previously reported [24,25]. After calculation of the 32 chemical descriptors, correlation coefficients between descriptors and correlation of the descriptors with the P-gp modulator category (substrate or inhibitor) were determined using SPSS statistics software version 23.0.0.3 (IBM, Armonk, NY: IBM Corp, USA). If the correlation coefficient between the P-gp modulator category (substrate or inhibitor) and a certain descriptor was below 0.1, this descriptor was omitted. Only descriptors correlating with the P-gp modulator (substrate or inhibitor) category above 0.1 were selected for further processing. As a next step, descriptors having a pairwise correlation coefficient to the P-gp modulator category lower than 0.9 were excluded [26]. By this strategy, relevant descriptors without an issue of over-fitting can be selected.

2.2. P-Glycoprotein Modulator Prediction Model Establishment

At first, a model, which can predict whether a given compound is a P-gp modulator, was built by using the compound list from Broccatelli et al. [27] After applying the descriptor selection criteria by considering the relevancy and over-fitting issues, “logP”, “H-donors”, “polar surface area”, “ligand efficiency dependent lipophilicity”, “molecular complexity”, “stereo centers”, “rotatable bonds”, “rings closures”, “aromatic rings”, “sp-3 atoms”, “amides”, “amines”, “alkyl-amines,” and “basic nitrogens” were considered for the preparation of the P-gp modulator/non-modulator prediction model. Various classification algorithms with the leave-one-out random sampling method were tested, i.e., k-Nearest Neighboring (kNN), Neural Network, Random Forest (RF), and Support Vector Machine (SVM). Receiver operating characteristic (ROC) curves are depicted in Figure 1. The receiver operating characteristic (ROC) curve plotted the true positive rate (= sensitivity) against the false positive rate (= 1-specificity). The RF algorithm performed better than the other classification algorithms both in learning and validation steps. The overall performance for the established model based on RF algorithm is summarized in Table 3. The establishment of the P-gp modulator/non-modulator and P-gp inhibitor/substrate prediction models were performed by using the machine learning software Orange (Ljubljana, Slovenia) [27].

![Figure 1. Receiver operating characteristic (ROC) curves of k Nearest Neighboring (kNN), Neural Network, Random Forest (RF), and Support Vector Machine (SVM) classification algorithms based on random leave-one-out sampling for the P-gp modulator/non-modulator prediction model for the learning step.](image-url)
Table 3. Performance of the P-gp modulator/non-modulator prediction model based on the RF classifier algorithm.

| Steps          | Sensitivity | Specificity | Overall Predictive Accuracy | Precision |
|----------------|-------------|-------------|----------------------------|-----------|
| Learning       | 0.938       | 0.969       | 0.953                      | 0.968     |
| External Validation | 0.938       | 0.938       | 0.938                      | 0.938     |

After applying the descriptor selection criteria by considering the relevancy and over-fitting issues, “logP”, “total surface area”, “shape index”, “molecular flexibility”, “rotatable bonds”, “aromatic rings”, “aromatic atoms”, “aromatic nitrogens”, “basic nitrogens”, “symmetric atoms”, and “acidic oxygens” were considered for P-gp inhibitor/substrate prediction model preparation. Various classification algorithms with the leave-one-out random sampling method were tested, i.e., kNN, Neural Network, RF, and SVM. The ROC curves are depicted in Figure 2. The RF algorithm performed better than the other classification algorithms. The overall performance for the established model is summarized in Table 4.

![ROC curves](image_url)

**Figure 2.** ROC curves of kNN, Neural Network, RF, and SVM classification algorithms based on random leave-one-out sampling for the P-gp inhibitor/substrate prediction model for the learning step.

Table 4. Performance of the P-gp inhibitor/substrate prediction model based on the RF classifier algorithm.

| Steps          | Sensitivity | Specificity | Overall Predictive Accuracy | Precision |
|----------------|-------------|-------------|----------------------------|-----------|
| Learning       | 0.750       | 0.700       | 0.725                      | 0.714     |
| External Validation | 0.786       | 0.833       | 0.800                      | 0.917     |

In order to evaluate the model performance further and select potential inhibitors, a P-gp modulator compound list consisting of 643 compounds from ChEMBL was used.

### 2.3. Molecular Docking

The recently published human P-gp structure was used (nanodisc reconstituted in complex with UIC2 fab and paclitaxel at the drug-binding pocket, PDB ID: 6QEX, in the absence of a lipid bilayer) [28]. The Fab chains were deleted. The bound ligands marked as “HETATM” including taxol were also deleted from the PDB structure file in order to prevent interference with molecular docking. The preparation of the final receptor structure as “.pdbqt” file was performed with Autodock tools 1.5.7. Selected compounds from inhibitor and substrate classes have been subjected to an automated and comprising molecular docking campaign by using the high-performance supercomputer MOGON (Johannes Gutenberg University, Mainz). Compound flexibilities were taken into account and a rigid receptor structure was used. At first, three independent screening of all 643 compounds from ChEMBL with Autodock Vina algorithm was performed by focusing on the drug-binding pocket of...
P-gp, where the majority of the known inhibitors and substrates bind to. The grid parameters are listed in Table 5.

Table 5. Grid parameters for molecular docking analyses on human P-gp.

|                  | x     | y     | z     |
|------------------|-------|-------|-------|
| Number of Points | 126   | 98    | 116   |
| Grid Center      | 168.614 | 166.372 | 162.000 |
| Grid Spacing (Å) | 0.375                        |

Afterward, the top 20 compounds in terms of binding energy yielded from both inhibitor and substrate predictions were selected for molecular docking. Each molecular docking was based on three independent dockings each consisting of 2,500,000 calculations. This means that each data point represents the mean value of 7,500,000 individual MOGON-based calculations. The Autodock 4 algorithm was used for defined molecular docking calculations on the drug-binding pocket of P-gp as described before [11], and Visual Molecular Dynamics (VMD) software (Theoretical and Computational Biophysics group at the Beckman Institute, University of Illinois at Urbana-Champaign) was used for the visualization of the docking poses. Estimated inhibition constants were calculated by the Autodock algorithm with the equation:

$$Ki = \exp\left(\frac{\Delta G}{RT}\right)$$

(1)

$Ki$ (M)

$\Delta G$ (cal/mol) = 1000 * LBE (lowest binding energy, kcal/mol)

$R$ (cal/mol-K); gas constant, 1.986 cal/mol-K

$T$ (K); room temperature, 298 K

2.4. Boxplot Analysis

The distribution of the values for the descriptors used for the P-gp inhibitor/substrate prediction model and the comparison for the predicted inhibitors and substrates among the ChEMBL P-gp modulator list were subjected to Boxplot analysis using Microsoft Excel 2019 (Microsoft, USA). Statistical significances were evaluated by the t-test (two-tailed, two-sample unequal variance).

3. Results

3.1. P-glycoprotein Modulator Predictions

The P-gp modulator/non-modulator prediction model was evaluated with the validation set as mentioned in the corresponding method part. The RF algorithm reached 0.938 for all parameters. The ChEMBL P-gp modulator list of 643 compounds was tested, and 641 out of 643 substances were correctly predicted as modulators.

The P-gp inhibitor/substrate prediction model with the ChEMBL P-gp modulator list of 643 compounds was evaluated. A total of 493 substances were predicted as inhibitors, and 150 compounds were predicted as substrates. Subjecting all compounds to Autodock Vina screening allowed to rank them according to their binding energies. The top 20 inhibitor predictions with strong interaction to P-gp are shown in Table 6. These inhibitors were selected for subsequent molecular docking. The top 20 substrate predictions with strong interaction to P-gp are shown in Table 7. These substrates were also selected substances for subsequent molecular docking. The complete predictions for all 493 inhibitors together with their binding affinities to P-gp are shown in Supplementary Table S1, while all predictions for the 150 substrates and their affinities to P-gp are listed in Supplementary Table S2. The average lowest binding energy (LBE) was -8.155 for the inhibitors and -9.289 for the substrates.
Table 6. Prediction of the top 20 P-gp inhibitors identified by the RF classification algorithm using the ChEMBL P-gp modulator list of 493 compounds. The results were validated by determining the binding affinities using Autodock VINA.

| Name             | ChEMBL ID      | Inhibitor Probability | Class      | VINA LBE (kcal/mol) |
|------------------|----------------|-----------------------|------------|---------------------|
| Karavoate P      | CHEMBL1641677  | 0.849                 | Synthetic  | −12.200 ± 1.212     |
| Tribenzoylbalsaminol F | CHEMBL1928854 | 0.549                 | Synthetic  | −12.033 ± 0.896     |
| Zosuquidar      | CHEMBL444172   | 0.513                 | Synthetic  | −11.967 ± 0.058     |
| Latilagascenes D | CHEMBL435917   | 0.566                 | Synthetic  | −11.700 ± 0.001     |
| Dihydrocytochalasin B | CHEMBL2074735 | 0.513                 | Synthetic  | −11.367 ± 0.231     |
| Jolkinoate I    | CHEMBL2315618  | 0.593                 | Synthetic  | −11.300 ± <0.001    |
| Karavoate K      | CHEMBL1641672  | 0.849                 | Synthetic  | −11.267 ± 0.493     |
| Fanchinin       | CHEMBL176045   | 0.586                 | Synthetic  | −11.233 ± 0.208     |
| Latilagascene I | CHEMBL511018   | 0.586                 | Synthetic  | −11.167 ± 0.058     |
| Karavoate L      | CHEMBL1641673  | 0.766                 | Synthetic  | −11.133 ± 0.808     |
| 3-Methylcholanthrene | CHEMBL40583  | 0.788                 | Synthetic  | −11.100 ± <0.001    |
| Lonafarnib       | CHEMBL298734   | 0.567                 | Synthetic  | −11.000 ± <0.001    |
| Karavoate N      | CHEMBL1641675  | 0.666                 | Synthetic  | −10.933 ± 0.058     |
| Tariquidar       | CHEMBL348475   | 0.619                 | Synthetic  | −10.933 ± 0.404     |
| Pimozide         | CHEMBL14123    | 0.517                 | Synthetic  | −10.900 ± 0.100     |
| Karavoate I      | CHEMBL1641670  | 0.766                 | Synthetic  | −10.767 ± 0.058     |
| Cryptotanshinone | CHEMBL187460   | 0.663                 | Natural    | −10.700 ± <0.001    |
| Jolkinoil B      | CHEMBL296419   | 0.577                 | Synthetic  | −10.667 ± 0.115     |
| Metergoline      | CHEMBL19215    | 0.732                 | Natural    | −10.600 ± <0.001    |

Table 7. Prediction of P-gp substrates identified by the RF classification algorithm using the ChEMBL P-gp modulator list of 150 compounds. The results were validated by determining the binding affinities using Autodock VINA.

| Name           | ChEMBL ID      | Substrate probability | Class     | VINA LBE (kcal/mol) |
|----------------|----------------|-----------------------|-----------|---------------------|
| Vindoline      | CHEMBL526546   | 0.771                 | Synthetic | −15.000 ± <0.001    |
| Cepharanthin   | CHEMBL2074948  | 0.614                 | Natural   | −12.600 ± <0.001    |
| Latilagascene G| CHEMBL448193   | 0.514                 | Synthetic | −12.300 ± <0.001    |
| Mk3207         | CHEMBL1910936  | 0.733                 | Synthetic | −12.167 ± 0.058     |
| Ergocristine   | CHEMBL446315   | 0.767                 | Natural   | −12.067 ± 0.058     |
| Cytochalasin E | CHEMBL494856   | 0.6                   | Natural   | −11.800 ± <0.001    |
| Jolkinoate L   | CHEMBL2315621  | 0.567                 | Synthetic | −11.533 ± 0.058     |
| Irinotecan     | CHEMBL481     | 0.967                 | Natural   | −11.400 ± 0.819     |
| Latilagascenes E| CHEMBL373511  | 0.614                 | Synthetic | −11.367 ± 0.116     |
| Dofequidar     | CHEMBL65067    | 0.583                 | Synthetic | −11.300 ± 0.001     |
| Acetyldigoxin  | CHEMBL2074725  | 0.708                 | Natural   | −11.233 ± 0.808     |
| Dihydroergocristine | CHEMBL601773 | 0.767                 | Natural   | −11.133 ± 0.666     |
| Telcagepant    | CHEMBL236593   | 0.517                 | Synthetic | −11.067 ± 0.058     |
| Ergotamine     | CHEMBL442     | 0.8                   | Natural   | −10.933 ± 0.058     |
Among the 493 inhibitor compounds were 117 natural products (= 23.7%), while all other compounds were of synthetic origin (Supplementary Table S1). The proportion of natural products was higher among the predicted P-gp substrates (69/150 = 46%) (Supplementary Table S2). This trend was even more apparent if we focused on the top 20 inhibitor or substrate compounds only (Tables 6 and 7). Here, 2/20 (= 10%) were predicted inhibitors, but 11/20 (= 55%) were predicted substrates, indicating that P-glycoprotein may expel natural xenobiotics from cells with higher probability.

3.2. Molecular Docking

After running the prediction model on the P-gp modulator list from ChEMBL and the Autodock VINA screening, the top 20 compounds from the inhibitor class and the top 20 compounds from the substrate class were selected for molecular docking analyses on human P-gp. The lowest binding energies (LBE) and predicted inhibition constants are listed in Table 8 for the inhibitors and Table 9 for the substrates.

Table 7. Cont.

| Name                | ChEMBL ID     | Substrate probability | Class      | VINA LBE (kcal/mol) |
|---------------------|---------------|-----------------------|------------|---------------------|
| Candesartan Cilexetil | CHEMBL1014   | 0.567                 | Synthetic  | −10.900 ± 0.200     |
| Digoxin             | CHEMBL1751   | 0.708                 | Natural    | −10.833 ± 1.097     |
| Bromocriptine       | CHEMBL493    | 0.767                 | Natural    | −10.800 ± 0.100     |
| Itrazole            | CHEMBL64391  | 0.564                 | Synthetic  | −10.700 ± 0.436     |
| Digitoxin           | CHEMBL254219 | 0.725                 | Natural    | −10.667 ± 0.462     |
| Paclitaxel          | CHEMBL428647 | 0.808                 | Natural    | −10.633 ± 0.462     |

Table 8. Lowest binding energies (LBE) and predicted inhibition constants obtained by molecular docking of the top 20 P-gp inhibitors.

| P-gp Inhibitor    | AutoDock LBE (kcal/mol) | Predicted Inhibition Constant (µM) |
|-------------------|-------------------------|------------------------------------|
| 3-Methylcholanthrene | −8.900 ± 0.001          | 0.300 ± <0.001                    |
| Astemizole        | −9.693 ± 0.047          | 0.079 ± 0.007                     |
| Cryptotanshinone  | −9.010 ± 0.001          | 0.251 ± <0.001                    |
| Dihydrocytochalasin B | −10.460 ± 0.020        | 0.0212 ± 0.001                    |
| Fanchinin         | −9.937 ± 0.067          | 0.0522 ± 0.006                    |
| Jolkinoate I      | −10.440 ± 0.200         | 0.0232 ± 0.008                    |
| Jolkinol B        | −10.250 ± 0.044         | 0.0307 ± 0.002                    |
| Karavoate I       | −12.310 ± 0.235         | 0.001 ± <0.001                    |
| Karavoate K       | −12.330 ± 0.213         | 0.001 ± <0.001                    |
| Karavoate L       | −12.807 ± 0.200         | 0.0004 ± <0.001                   |
| Karavoate N       | −12.160 ± 0.560         | 0.002 ± 0.001                     |
| Karavoate P       | −13.537 ± 0.605         | 0.0002 ± <0.001                   |
| Latilagascene I   | −11.147 ± 0.561         | 0.009 ± 0.009                     |
| Latilagascenes D  | −12.220 ± 0.370         | 0.001 ± 0.001                     |
| Lonafarnib        | −11.433 ± 0.087         | 0.004 ± 0.001                     |
| Metergoline       | −9.737 ± 0.029          | 0.073 ± 0.004                     |
| Pimozide          | −10.220 ± 0.324         | 0.031 ± 0.025                     |
| Tariquidar        | −11.273 ± 0.274         | 0.006 ± 0.002                     |
| Tribenzoylbalsaminol F | −12.403 ± 0.118   | 0.001 ± <0.001                    |
| Zosuquidar        | −11.257 ± 0.361         | 0.006 ± 0.004                     |
| Elacridar (positive control) | −11.093 ± 0.361 | 0.008 ± 0.004                    |
Table 9. Lowest binding energies (LBE) and predicted inhibition constants obtained by molecular docking of the top 20 P-gp substrates.

| P-gp Substrate   | AutoDock LBE (kcal/mol) | Predicted Inhibition Constant (µM) |
|------------------|-------------------------|-----------------------------------|
| Acetyldigoxin    | −11.767 ± 0.480         | 0.003 ± 0.002                     |
| Bromocriptine    | −12.360 ± 1.02          | 0.002 ± 0.001                     |
| Candesartan Cilexetil | −11.153 ± 0.370   | 0.007 ± 0.004                     |
| Cepharanthin     | −10.753 ± 0.006         | 0.013 ± <0.001                    |
| Cytochalasin E   | −10.957 ± 0.006         | 0.093 ± 0.001                     |
| Digitoxin        | −11.390 ± 0.517         | 0.006 ± 0.004                     |
| Digoxin          | −11.500 ± 0.151         | 0.004 ± 0.001                     |
| Dihydroergocristine | −11.670 ± 0.056    | 0.003 ± <0.001                    |
| Dofequidar       | −10.970 ± 0.351         | 0.010 ± 0.006                     |
| Ergocristine     | −12.407 ± 0.012         | 0.001 ± <0.001                    |
| Ergotamine       | −11.227 ± 0.150         | 0.006 ± 0.001                     |
| Irinotecan       | −11.380 ± 0.020         | 0.005 ± <0.001                    |
| Itrazole         | −10.843 ± 0.186         | 0.012 ± 0.003                     |
| Jolkinoate L     | −10.643 ± 0.681         | 0.022 ± 0.016                     |
| Latilagescenes E | −11.770 ± 0.185         | 0.002 ± 0.001                     |
| Latilagescene G  | −12.500 ± 0.316         | 0.001 ± <0.001                    |
| Mk-3207          | −11.650 ± 0.020         | 0.003 ± <0.001                    |
| Paclitaxel       | −9.607 ± 0.359          | 0.103 ± 0.065                     |
| Telcagepant      | −9.333 ± 0.021          | 0.144 ± 0.005                     |
| Vindoline        | −7.337 ± 0.211          | 4.363 ± 1.389                     |
| Doxorubicin (positive control) | −11.070 ± 0.135 | 0.008 ± 0.002                     |

The negative control compounds (oxprenolol, promazine, riluzole) revealed weaker interaction with P-gp (Table 10) and slightly different docking pose as well (Figure 3).

As can be seen in Figure 4, the predicted inhibitors possessed similar docking poses as elacridar at the drug-binding pocket of P-gp. Similar results were observed for the substrates: The predicted substrates revealed similar docking poses as doxorubicin. Hence, these results validated the precision and reliability of the model.

Table 10. Lowest binding energies (LBE) and predicted inhibition constants obtained by molecular docking of the non-modulators.

| P-gp Inhibitor | AutoDock LBE (kcal/mol) | Predicted Inhibition Constant (µM) |
|----------------|-------------------------|-----------------------------------|
| Oxprenolol     | −5.743 ± 0.398          | 70.273 ± 40.057                   |
| Promazine      | −6.933 ± 0.021          | 8.273 ± 0.262                     |
| Riluzole       | −5.380 ± 0.010          | 114.080 ± 2.326                   |
Predicted inhibitors and substrates interact with P-gp significantly stronger than the negative control compounds. This is clear both from the binding energies and predicted inhibition constants. Binding energies of non-modulators are within $-5.380$ (piluzole) to $-6.933$ (promazine) kcal/mol and the predicted inhibition constants are within $8.273$–$114.080$ µM, whereas binding energies for the predicted substrates are within $-7.337$ (vindoline) to $-12.500$ (latilagescene G) and for the predicted inhibitors $-8.900$ (3-methylcholanthrene) to $-13.537$ (karavante P). Predicted inhibition constants for the predicted substrates are within $0.001$–$4.363$ and for the predicted inhibitors $0.0002$–$0.300$ µM. Docking pose of the negative control compounds differs from that of inhibitors and substrates. Overall, it can
be speculated that the predicted inhibitors interact with P-gp stronger than the predicted substrates
and the non-modulators are making weak interactions with P-gp and they bind to a different site.

The distribution of the values for the descriptors used to build the model and the comparison
for the predicted inhibitors and substrates in terms of those descriptor values were performed with
Boxplot analysis. As can be seen from Figure 5, the inhibitors revealed significantly different values for
all descriptors except logP and acidic oxygens. The average values of descriptors for inhibitors and
substrates are listed in Table 11.

Table 11. Average values of descriptors for inhibitors and substrates

| Descriptor           | Inhibitor (mean ± SD) | Substrate (mean ± SD) |
|----------------------|-----------------------|-----------------------|
| logP                 | 3.498 ± 2.464         | 3.134 ± 2.962         |
| Total surface area   | 311.199 ± 188.142     | 461.870 ± 286.187     |
| Shape index          | 0.529 ± 0.125         | 0.429 ± 0.081         |
| Molecular flexibility| 0.395 ± 0.141         | 0.332 ± 0.114         |
| Rotatable bonds      | 6.799 ± 12.158        | 9.818 ± 11.778        |
| Aromatic rings       | 1.450 ± 1.168         | 1.918 ± 1.330         |
| Aromatic atoms       | 8.237 ± 6.470         | 10.759 ± 7.098        |
| Symmetric atoms      | 2.649 ± 3.637         | 3.582 ± 4.477         |
| Aromatic nitrogens   | 0.301 ± 0.772         | 0.559 ± 1.141         |

Figure 5. Boxplot analysis of the descriptors used for the model and comparison of the predicted
inhibitors and substrates.
Table 11. Average values of descriptors for inhibitors and substrates.

| Descriptor          | Inhibitor       | Substrate       |
|---------------------|-----------------|-----------------|
| cLogP               | 3.498 ± 2.464   | 3.134 ± 2.962   |
| Total surface area  | 311.199 ± 188.142 | 461.870 ± 286.187 |
| Shape index         | 0.529 ± 0.125   | 0.429 ± 0.081   |
| Molecular flexibility| 0.395 ± 0.141   | 0.332 ± 0.114   |
| Rotatable bonds     | 6.799 ± 12.158  | 9.818 ± 11.778  |
| Aromatic rings      | 1.450 ± 1.168   | 1.918 ± 1.330   |
| Aromatic atoms      | 8.237 ± 6.470   | 10.759 ± 7.098  |
| Symmetric atoms     | 2.649 ± 3.637   | 3.582 ± 4.477   |
| Aromatic nitrogens  | 0.301 ± 0.772   | 0.559 ± 1.141   |
| Basic nitrogens     | 0.441 ± 0.625   | 0.659 ± 0.762   |
| Acidic oxygens      | 0.117 ± 0.361   | 0.171 ± 0.462   |

4. Discussion

In the present study, we utilized machine learning methods based on leave-one-out random sampling in order to develop a P-gp modulator prediction platform by using chemical descriptors. The main focus was to predict whether a given compound can behave as substrate or inhibitor of P-gp. The RF classification algorithm (AUC: 0.774) outperformed the other tested algorithms (kNN—0.676, Neural Network—0.745, SVM—0.720). Performance scores for the external validation set were even higher than the learning set with better sensitivity (0.786 vs. 0.750), specificity (0.833 vs. 0.700), overall prediction accuracy (0.800 vs. 0.725), and precision (0.917 vs. 0.714). Further testing with the P-gp modulator list from ChEMBL yielded promising results with accurate predictions. Four compounds from inhibitor and four compounds from substrate prediction list were selected for molecular docking analyses. Validations with molecular docking on a recently released human P-gp structure were performed in terms of binding energy and docking poses by including known inhibitor (elacridar) and substrate (doxorubicin) as controls. Curcumin, miconazole, tacrolimus, and venlafaxine revealed a similar docking pose at the drug-binding pocket of P-gp with comparable binding energies with that of elacridar. MK-3207, rifampin, vindoline, and voacamine revealed similar docking poses and comparable binding energy with those of doxorubicin. Overall, the precision and reliability of the model were further confirmed.

Machine learning and artificial intelligence attracted increasing interest in the drug discovery area [18,29,30], and utilizing these methods possess great potential for drug discovery, as they save time and costs during the preclinical steps. The RF algorithm depends on multiple decision trees that are built based on the training data, and a majority voting scheme is used to make classification or regression predictions [31]. RF application to drug discovery has been recently reported, and it outperformed other algorithms such as SVM and NN in terms of feature selection [32].

There are various studies in the literature that utilized machine-learning strategies focusing on P-gp. One study pointed out a P-gp substrate prediction model based on RF algorithm to estimate transport potential for central nervous system drugs, accuracy lies between 0.713 and 0.846 whereas precision is between 0.633 and 0.777 [33]. Our P-gp modulator prediction model involves an accuracy of 0.953 for the learning set and 0.938 for the validation set, and our P-gp inhibitor prediction model has an accuracy value of 0.725 for the learning set and 0.800 for the validation set. In terms of precision, our models also perform better. Modulator prediction model involves a precision of 0.968 for the learning set and 0.938 for the validation set. Inhibitor prediction model has a 0.714 precision for the learning set and 0.917 for the validation set. Similarly, a P-gp substrate efflux ratio prediction model has been recently reported based on SVM algorithm [34]. The affinities of flavonoids to P-gp have been evaluated with an SVM-based model and a high correlation with the experimental data has been achieved [35]. Another study involving P-gp inhibitor prediction was performed for chalcone derivatives and selected inhibitor candidates were analyzed in terms of their docking pose on a homology model of human P-gp [36]. The prediction of blood–brain barrier permeability mechanism
of central nervous system drugs has been utilized with an SVM-based model [37]. Binding pattern prediction based on pharmacophore ensemble/SVM method for potential P-gp inhibitors was also recently reported [38]. Another SVM-based model coupled with molecular docking aimed to predict whether a given compound may act as P-gp substrate, the accuracy lies between 0.750 and 0.800, specificity between 0.750 and 0.810, and sensitivity between 0.740 and 0.790 [39]. Our modulator prediction model outperforms that model in all those parameters. Our inhibitor prediction model outperforms in the validation set. Similarly, in 2004, SVM-based P-gp substrate prediction model was reported; sensitivity was 0.812, specificity was 0.792, and accuracy was 0.794 [40]. Our modulator prediction model outperforms that model in all those parameters. Our inhibitor prediction model outperforms in the validation set for the specificity and accuracy parameters. In general, these previously published studies have certain disadvantages, e.g., low performance scores in terms of prediction, focusing on only P-gp substrate prediction or molecular docking with homology models but not crystal structures. Our model is superior compared to the previously published studies for several reasons. It is based on leave-one-out random sampling RF algorithm, focused on both natural as well as synthetic compounds, has high sensitivity, specificity, predictive accuracy, and precision to predict at first P-gp modulator/non-modulator and as a next step to predict P-gp substrate/inhibitor depending on various chemical descriptors, and it was coupled with molecular docking using the recently released crystal structure of human P-gp. The fact that predictions on the P-gp modulator list of compounds from ChEMBL was validated with accurate molecular docking results was also advantageous for our model. Furthermore, after the initial compound screening, selected inhibitors revealed similar docking poses as elacridar (as positive control for an inhibitor) and selected substrates revealed similar docking poses as doxorubicin (as positive control for a substrate). Non-modulators have significantly weaker interaction with P-gp and they bind to a slightly different position. Overall, those observations provide further clues for the reliability of the prediction model.

Selected inhibitors and substrates after the virtual screening are supported by literature; astemizole [41], cryptotanshinone [42], dihydrocytochalasin B [43], jolkinol B [44], latilagascenes D [45], lonafarnib [46], tariquidar [12], zosuquidar [47], acetildigitoxin [48], bromocriptine [49], candesartan cilexetil [50], cepharanthin [51], cytochalasin E [52], digitoxin [53], digoxin [54], dihydroergosrictine [55], dof equidar [56], ergocristine [55], irinotecan [57], latilagascenes E [45], MK-3207 [58], paclitaxel [59], vindoline [60].

Many cancer types involve P-gp overexpression, which is associated with increased efflux of established anticancer drugs and natural cytotoxic products out of cancer cells. This phenomenon represents a major drawback of cancer chemotherapy with limitations in killing tumor populations due to MDR [61,62]. P-gp overexpression is indeed one of the main reasons for MDR and thus inadequate chemotherapy success rate. Targeting P-gp is critical to achieve high success rates for chemotherapy, therefore, identification of novel P-gp inhibitors is critical in that regard.

Our prediction platform for P-gp modulators facilitates to predict whether a given compound can behave as a substrate or an inhibitor of P-gp. The selection of potential inhibitors can be further validated by molecular docking and the comparison of the binding energy and docking pose with those of known P-gp inhibitors. As a next step in the future, our model may be helpful to identify potential novel P-gp inhibitors and to develop effective chemotherapy strategies involving combination therapy with targeted chemotherapy drugs and identified P-gp inhibitors.

5. Conclusion

In the present study, we established P-gp modulator/non-modulator and inhibitor/substrate prediction models based on the RF algorithm and leave-one-out random sampling. Validation with molecular docking was performed. The identification of novel P-gp inhibitors is critical to overcome MDR and to achieve better chemotherapy strategies. This model can predict whether a given compound can behave as substrate or inhibitor of P-gp, and will be, thus, helpful to identify potential P-gp inhibitors.
Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4409/8/10/1286/s1, Table S1: Prediction of P-gp inhibitors identified by the random forest classification algorithm using the ChEMBL P-gp modulator list of 493 compounds, Table S2: Prediction of P-glycoprotein substrates identified by the RF classification algorithm using the ChEMBL P-gp modulator list of 150 compounds.

Author Contributions: Conceptualization, O.K.; Investigation, O.K., T.E.; Methodology, O.K.; Supervision, T.E.; Writing—original draft, O.K.; Writing—review & editing, T.E.

Funding: O.K. is funded by intramural funds at the Johannes Gutenberg University Mainz, Germany.

Acknowledgments: Parts of this research were conducted using the supercomputer Mogon and/or advisory services offered by Johannes Gutenberg University Mainz (hpc.uni-mainz.de), which is a member of the AHRP (Alliance for High-Performance Computing in Rhineland Palatinate, www.ahrp.info) and the Gauss Alliance e.V. The authors gratefully acknowledge the computing time granted on the supercomputer Mogon at Johannes Gutenberg University Mainz (hpc.uni-mainz.de).

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ABC ATP binding cassette
AUC area under the curve
kNN k-nearest neighboring
MDR multidrug resistance
P-gp P-glycoprotein
RF random forest
ROC receiver operating characteristic
SVM support vector machine

References

1. Efferth, T.; Zeino, M.; Volm, M. Modulation of P-glycoprotein-mediated multidrug resistance by synthetic and phytochemical small molecules, monoclonal antibodies, and therapeutic nucleic acids. In Resistance to Targeted ABC Transporters in Cancer; Efferth, T., Ed.; Springer International Publishing: Cham, Switzerland, 2015; pp. 153–181. [CrossRef]
2. Krech, T.; Scheuerer, E.; Geffers, R.; Kreipe, H.; Lehmann, U.; Christgen, M. ABCB1/MDR1 contributes to the anticancer drug-resistant phenotype of IPH-926 human lobular breast cancer cells. Cancer Lett. 2012, 315, 153–160. [CrossRef] [PubMed]
3. Burger, H.; Nooter, K. Pharmacokinetic resistance to imatinib mesylate—Role of the ABC drug pumps ABCG2 (BCRP) and ABCB1 (MDR1) in the oral bioavailability of imatinib. Cell Cycle 2004, 3, 1502–1505. [CrossRef] [PubMed]
4. Efferth, T. Inhibition of P-glycoprotein at the blood brain barrier by phytochemicals derived from traditional Chinese medicine. Planta Medica 2009, 75, SL3. [CrossRef]
5. Kuete, V.; Fouotsa, H.; Mbaveng, A.T.; Wiench, B.; Nkengfack, A.E.; Efferth, T. Cytotoxicity of a naturally occurring furoquinoline alkaloid and four acridone alkaloids towards multi-factorial drug-resistant cancer cells. Phytotherapy 2015, 22, 946–951. [CrossRef] [PubMed]
6. Kadioglu, O.; Cao, J.; Kosyakova, N.; Mrasek, K.; Liehr, T.; Efferth, T. Genomic and transcriptomic profiling of resistant CEM/ADR-5000 and sensitive CCRF-CEM leukaemia cells for unravelling the full complexity of multi-factorial multidrug resistance. Sci. Rep. 2016, 6, 36754. [CrossRef]
7. Kuete, V.; Saeed, M.E.M.; Kadioglu, O.; Bortzler, J.; Khalid, H.; Greten, H.J.; Efferth, T. Pharmacogenomic and molecular docking studies on the cytotoxicity of the natural steroid wortmannin against multidrug-resistant tumor cells. Phytomedicine 2015, 22, 120–127. [CrossRef]
8. Efferth, T.; Osieka, R. Clinical Relevance of the Mdr-1 gene and its gene-product, P-glycoprotein for cancer chemotherapy—a metaanalysis. Tumordiagn Ther. 1993, 14, 238–243.
9. Efferth, T. The human ATP-binding cassette transporter genes: From the bench to the bedside. Curr. Mol. Med. 2001, 1, 45–65. [CrossRef]
10. Gillet, J.P.; Efferth, T.; Remacle, J. Chemotherapy-induced resistance by ATP-binding cassette transporter genes. Biochim. Biophys. Acta Rev. Cancer 2007, 1775, 237–262. [CrossRef]
11. Kadioglu, O.; Saeed, M.E.M.; Valoti, M.; Frosini, M.; Sgaragli, G.; Efferth, T. Interactions of human P-glycoprotein transport substrates and inhibitors at the drug binding domain: Functional and molecular docking analyses. Biochem. Pharmacol. 2016, 104, 42–51. [CrossRef]

12. Srinivas, N.R. Understanding the role of tariquidar, a potent Pgp inhibitor, in combination trials with cytotoxic drugs: What is missing? Cancer Chemother. Pharmacol. 2016, 78, 1097–1098. [CrossRef] [PubMed]

13. Kelly, R.J.; Draper, D.; Chen, C.C.; Robey, R.W.; Figg, W.D.; Piekarz, R.L.; Chen, X.; Gardner, E.R.; Balis, F.M.; Venkatesan, A.M.; et al. A pharmacodynamic study of docetaxel in combination with the P-glycoprotein antagonist tariquidar (XR9576) in patients with lung, ovarian, and cervical cancer. Clin. Cancer Res. 2011, 17, 569–580. [CrossRef] [PubMed]

14. Abraham, J.; Edgerly, M.; Wilson, R.; Chen, C.; Ratt, A.; Bakke, S.; Robey, R.; Dwyer, A.; Goldspiel, B.; Balis, F.; et al. A phase I study of the P-glycoprotein antagonist tariquidar in combination with vinorelbine. Clin. Cancer Res. 2009, 15, 3574–3582. [CrossRef] [PubMed]

15. Fox, E.; Widemann, B.C.; Pastakia, D.; Chen, C.C.; Yang, S.X.; Cole, D.; Balis, F.M. Pharmacokinetic and pharmacodynamic study of tariquidar (XR9576), a P-glycoprotein inhibitor, in combination with doxorubicin, vinorelbine, or docetaxel in children and adolescents with refractory solid tumors. Cancer Chemother. Pharmacol. 2015, 76, 1273–1283. [CrossRef] [PubMed]

16. Zhang, Y.M.; Wang, Y.C.; Zhou, W.N.; Fan, Y.R.; Zhao, J.N.; Zhu, L.; Lu, S.; Lu, T.; Chen, Y.D.; Liu, H.C. A combined drug discovery strategy based on machine learning and molecular docking. Chem. Biol. Drug Des. 2019, 93, 685–699. [CrossRef] [PubMed]

17. Zoffmann, S.; Vercruysse, M.; Benmansour, F.; Maunz, A.; Wolf, L.; Marti, R.B.; Heckel, T.; Ding, H.Y.; Truong, H.H.; Prummer, M.; et al. Machine learning-powered antibiotics phenotypic drug discovery. Sci. Rep. 2019, 9, 5013. [CrossRef]

18. Vanamethevan, J.; Clark, D.; Czodrowski, P.; Dunham, I.; Ferran, E.; Lee, G.; Li, B.; Madabhushi, A.; Shah, P.; Spitzer, M.; et al. Applications of machine learning in drug discovery and development. Nat. Rev. Drug Discov. 2019, 18, 463–477. [CrossRef]

19. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs from 1981 to 2014. J. Nat. Prod. 2016, 79, 629–661. [CrossRef]

20. Saeed, M.; Kadioglu, O.; Khalid, H.; Sugimoto, Y.; Efferth, T. Activity of the dietary flavonoid, apigenin, against multidrug-resistant tumor cells as determined by pharmacogenomics and molecular docking. J. Nutr. Biochem. 2015, 26, 44–56. [CrossRef]

21. Seo, E.J.; Kuete, V.; Kadioglu, O.; Krusche, B.; Schroder, S.; Greten, H.J.; Arend, J.; Lee, I.S.; Efferth, T. Antiangiogenic activity and pharmacogenomics of medicinal plants from traditional korean medicine. Evidence-Based Complement. Altern. Med. 2013, 131306. [CrossRef]

22. Broccatelli, F. QSAR models for P-glycoprotein transport based on a highly consistent data set. J. Chem. Inf. Model. 2012, 52, 2462–2470. [CrossRef] [PubMed]

23. Zeino, M.; Saeed, M.E.M.; Kadioglu, O.; Efferth, T. The ability of molecular docking to unravel the controversy and challenges related to P-glycoprotein-a well-known, yet poorly understood drug transporter. Investig. New Drugs 2014, 32, 618–625. [CrossRef] [PubMed]

24. Sander, T.; Freyss, J.; von Korff, M.; Rufener, C. Data Warrior: An open-source program for chemistry aware data visualization and analysis. J. Chem. Inf. Model. 2015, 55, 460–473. [CrossRef] [PubMed]

25. Lopez-Lopez, E.; Naveja, J.J.; Medina-Franco, J.L. DataWarrior: An evaluation of the open-source drug discovery tool. Expert Opin. Drug Discov. 2019, 14, 335–341. [CrossRef]

26. Cai, C.P.; Fang, J.S.; Guo, P.F.; Wang, Q.; Hong, H.X.; Mosleh, J.; Cheng, F.X. In silico pharmacoepidemiologic evaluation of drug-induced cardiovascular complications using combined classifiers. J. Chem. Inf. Model. 2018, 58, 943–956. [CrossRef]

27. Demsar, J.; Curk, T; Erjavec, A.; Gorup, C.; Holecvar, T; Miletinovic, M.; Mozina, M.; Polajnar, M.; Toplak, M.; Staric, A.; et al. Orange: Data mining toolbox in python. J. Mach. Learn. Res. 2013, 14, 2349–2353.

28. Alam, A.; Kowal, J.; Broude, E.; Roninson, I.; Locher, K.P. Structural insight into substrate and inhibitor discrimination by human P-glycoprotein. Science 2019, 363, 753–756. [CrossRef]

29. Lo, Y.C.; Rensi, S.E.; Torng, W.; Altman, R.B. Machine learning in chemoinformatics and drug discovery. Drug Discov. Today 2018, 23, 1538–1546. [CrossRef]

30. Zhang, L.; Tan, J.J.; Han, D.; Zhu, H. From machine learning to deep learning: Progress in machine intelligence for rational drug discovery. Drug Discov. Today 2017, 22, 1680–1685. [CrossRef]
31. Schierz, A.C. Virtual screening of bioassay data. *J. Cheminformatics* **2009**, *1*, 21. [CrossRef]
32. Cano, G.; García-Rodriguez, J.; García-Garcia, A.; Pérez-Sanchez, H.; Benediktsson, J.A.; Thapa, A.; Barr, A. Automatic selection of molecular descriptors using random forest: Application to drug discovery. *Expert Syst. Appl.* **2017**, *72*, 151–159. [CrossRef]
33. Ohashi, R.; Watanabe, R.; Esaki, T.; Taniguchi, T.; Torimoto-Katori, N.; Watanabe, T.; Ogasawara, Y.; Takahashi, T.; Tsukimoto, M.; Mizuguchi, K. Development of simplified in vitro P-glycoprotein substrate assay and in silico prediction models to evaluate transport potential of P-glycoprotein. *Mol. Pharm.* **2019**, *16*, 1851–1863. [CrossRef] [PubMed]
34. Chen, C.; Lee, M.H.; Weng, C.F.; Leong, M.K. Theoretical prediction of the complex P-glycoprotein substrate eflux based on the novel hierarchical support vector regression scheme. *Molecules* **2018**, *23*, 1820. [CrossRef] [PubMed]
35. Cui, Y.; Chen, Q.G.; Li, Y.X.; Tang, L. A new model of flavonoids affinity towards P-glycoprotein: Genetic algorithm-support vector machine with features selected by a modified particle swarm optimization algorithm. *Arch. Pharmacal. Res.* **2017**, *40*, 214–230. [CrossRef] [PubMed]
36. Ngo, T.D.; Tran, T.D.; Le, M.T.; Thai, K.M. Computational predictive models for P-glycoprotein inhibition of in-house chalcone derivatives and drug-bank compounds. *Mol. Divers.* **2016**, *20*, 945–961. [CrossRef] [PubMed]
37. Jiang, L.D.; Chen, J.H.; He, Y.S.; Zhang, Y.L.; Li, G.Y. A method to predict di...
49. Vautier, S.; Lacomblez, L.; Chacun, H.; Picard, V.; Gimenez, F.; Farinotti, R.; Fernandez, C. Interactions between the dopamine agonist, bromocriptine and the efflux protein, P-glycoprotein at the blood-brain barrier in the mouse. *Eur. J. Pharm. Sci.* 2006, 27, 167–174. [CrossRef]

50. Zhou, L.J.; Chen, X.P.; Gu, Y.Q.; Liang, J.Y. Transport Characteristics of Candesartan in Human Intestinal Caco-2 Cell Line. *Biopharmacy and Drug Disposal* 2009, 30, 259–264. [CrossRef]

51. Koizumi, S.; Konishi, M.; Ichihara, T.; Wada, H.; Matsukawa, H.; Goi, K.; Mizutani, S. Flow Cytometric functional analysis of multidrug-resistance by Flu-3—A comparison with rhodamine-123. *Eur. J. Cancer* 1995, 31a, 1682–1688. [CrossRef]

52. Zilfou, J.T.; Smith, C.D. Differential interactions of cytochalasins with P-glycoprotein. *Oncol. Res.* 1995, 7, 435–443. [PubMed]

53. Rebbeor, J.F.; Senior, A.E. Effects of cardiovascular drugs on ATPase activity of P-glycoprotein in plasma membranes and in purified reconstituted form. *Biophys. Acta (BBA) Biomembr.* 1998, 1369, 85–93. [CrossRef]

54. Yamazaki, S.; Costales, C.; Lazzaro, S.; Eatemadpour, S.; Kimoto, E.; Varma, M.V. Physiologically-based pharmacokinetic modeling approach to predict rifampin-mediated intestinal P-glycoprotein induction. *CPT: Pharmacometrics Syst. Pharmacol.* 2019, 8, 634–642. [CrossRef] [PubMed]

55. Yasuda, K.; Lan, L.B.; Sanglard, D.; Furuya, K.; Schuetz, J.D.; Schuetz, E.G. Interaction of cytochrome P450 3A inhibitors with P-glycoprotein. *J. Pharmacol. Exp. Ther.* 2002, 303, 323–332. [CrossRef]

56. Takeshita, H.; Kusuzaki, K.; Tsuji, Y.; Hirata, M.; Hashiguchi, S.; Nakamura, S.I.; Murata, H.; Ashihara, T.; Hirasawa, Y. Avoidance of doxorubicin resistance in osteosarcoma cells using a new quinoline derivative, MS-209. *Anticancer. Res.* 1998, 18, 739–742.

57. Luo, F.R.; Paranjpe, P.V.; Guo, A.; Rubin, E.; Sinko, P. Intestinal transport of irinotecan in Caco-2 cells and MDCK II cells overexpressing efflux transporters PGP, cMOAT, and MRP1. *Drug Metab. Dispos.* 2002, 30, 763–770. [CrossRef]

58. Salvatore, C.A.; Moore, E.L.; Calamari, A.; Cook, J.J.; Michener, M.S.; O’Malley, S.; Miller, P.J.; Sur, C.; Williams, D.L.; Zeng, Z.Z.; et al. Pharmacological properties of MK-3207, a potent and orally active calcitonin gene-related peptide receptor antagonist. *J. Pharmacol. Exp. Ther.* 2010, 333, 152–160. [CrossRef]

59. Nemcova-Furstova, V.; Kopperova, D.; Balusikova, K.; Ehrlichova, M.; Brynychova, V.; Vaclavikova, R.; Daniel, P.; Soucek, P.; Kovar, J. Characterization of acquired paclitaxel resistance of breast cancer cells and involvement of ABC transporters. *Toxicol. Appl. Pharmacol.* 2016, 310, 215–228. [CrossRef]

60. Shepard, R.L.; Winter, M.A.; Hsaio, S.C.; Pearce, H.L.; Beck, W.T.; Dantzig, A.H. Effect of modulators on the ATPase activity and vanadate nucleotide trapping of human P-glycoprotein. *Biochem. Pharmacol.* 1998, 56, 719–727. [CrossRef]

61. Eadie, L.N.; Hughes, T.P.; White, D.L. ABCB1 Overexpression is a key initiator of resistance to tyrosine kinase inhibitors in CML cell lines. *PLoS ONE* 2016, 11, e0161470. [CrossRef]

62. Nemethova, V.; Razga, F. Overexpression of ABCB1 as prediction marker for CML: How close we are to translation into clinics? *Leukemia* 2017, 31, 266–267. [CrossRef] [PubMed]