Article

QSAR regression models for predicting HMG-CoA reductase inhibition based on MACCS molecular fingerprints and virtual screening of natural products

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**Abstract:**

**Background/Objectives**: HMG-CoA reductase is an enzyme that regulates the initial stage of cholesterol synthesis and its inhibitors are widely used in the treatment of cardiovascular diseases.

**Methods**: We have created a set of quantitative structure-activity relationship (QSAR) models for human HMG-CoA reductase inhibitors using nested cross-validation as the primary validation method. To develop the QSAR models, we employed various machine learning regression algorithms, feature selection methods, and fingerprints or descriptor datasets.

**Results**: We built and evaluated a total of 300 models, selecting 21 that demonstrated good performance (coefficient of determination, R2 ≥ 0.70 or concordance correlation coefficient, CCC ≥ 0.85). Six of these top-performing models met both performance criteria and were used to construct five ensemble models. We identified the descriptors most important in explaining HMG-CoA inhibition for each of the six best-performing models. We used the top models to search through over 220,000 chemical compounds from a large database (ZINC 15) for potential new inhibitors. Only a small fraction (237 out of approximately 220,000 compounds) had reliable predictions with mean pIC50 values ≥8 (IC50 values ≤10 nM). Our svm-based ensemble model predicted IC50 values <10 nM for roughly 0.08% of the screened compounds. We have also illustrated the potential applications of these QSAR models in understanding the cholesterol-lowering activities of herbal extracts, such as those reported for an extract prepared from the *Iris × germanica* rhizome.

**Conclusions**: Our QSAR models can accurately predict human HMG-CoA reductase inhibitors, having the potential to accelerate the discovery of novel cholesterol-lowering agents and may also be applied to understand the mechanisms underlying the reported cholesterol-lowering activities of herbal extracts.

**Keywords:** HMG-CoA reductase; QSAR; statins; nested cross-validation; virtual screening; *Iris germanica*; machine learning; feature selection; mlr3; MACCS fingerprints; molecular descriptors.

1. Introduction

The incidence of atherosclerotic cardiovascular disease is on the rise and continues to rank as the top cause of death and disability in industrialized countries. Atherosclerosis can be slowed or even reversed with the use of lipid-lowering agents when the medicines are administered in appropriate regimens, while the plaque is still immature and has not become calcified or fibrotic [1]. Evidence from both primary and secondary prevention studies shows that HMG-CoA reductase inhibitors (also known as statins) lessen the risk of atherosclerotic cardiovascular disease, making them the first-line lipid-lowering agents recommended by various national and international clinical guidelines [2]. HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase) is an enzyme that catalyzes an initial stage in the biosynthesis of cholesterol. This particular step is the one controlling the overall speed of the entire sequence of reactions involved in cholesterol synthesis [3]. Besides their main effects on HMG-CoA reductase, such inhibitors appear to have a large number of pleiotropic effects, providing cardiovascular protection independent of their effect on cholesterol, by preventing the formation of intermediates in the cholesterol biosynthetic pathway. These effects result in an inhibition of post-translational modifications of intracellular proteins. These changes, in turn, have downstream effects on endothelial, inflammatory, and smooth muscle cells [4]. The pleiotropic effects of statins and their potential therapeutic uses (related to the cholesterol inhibition or their pleiotropic effects) seem to be broad, from anti-inflammatory and immunomodulatory activities [5] to neuroprotective effects [6,7], from anti-tumorigenic and anti-metastatic actions [8,9] to protection against aging [10], from preventing or reducing the risk of osteoporosis [11] to certain effects on the endocrine system [12]. This topic, however, remains controversial, and the true impact of the reduction in these intermediates has not been fully clarified because it frequently corresponds to a simultaneous fall in cholesterol [4].

The currently available statins differ widely in their solubility and pharmacokinetic properties. Some are rather lipophilic (simvastatin, fluvastatin, lovastatin, pitavastatin, and atorvastatin), can easily penetrate biological membranes and tend to be more widely distributed in the body. Others, like pravastatin and a lesser extent rosuvastatin, are more hydrophilic. They stay connected to the polar surface of the membrane and need protein transporters to get into the cell. It is thought that because they are not as widely distributed, they might have less pleiotropic effects. [13]. Whereas approved statins seem often to be similar in their efficacy and safety, there are data suggesting that different statins have different safety profiles (with respect to their muscle-related side effects [14], liver toxicity [15,16], diabetes-risk [17], Alzheimer disease risk [18], drug interactions, etc. [19]) and different efficacy [20]. Therefore, developing new HMG-CoA reductase inhibitors could result in statins with improved or modified efficacy and safety..

QSAR is a computational approach that is based on building models describing the relationship between the biological activity and certain structural properties (descriptors) of ligands that bind to a specific biologic target (or who have a specific biological effect) [21]. Over time, two primary approaches to QSAR have emerged, based on the methods used to build the models. A first, more traditional one, is based on models that are often straightforward, linear, and may be interpreted in terms of physicochemical concepts. A second approach is based on the utilization of machine learning techniques, which are more suited for predicting the relationship between structure and activity in extensive datasets with significant chemical variability [22]. Molecular descriptors can capture broad categories of molecule structure information, such as bulk characteristics, substructure frequency, or more complicated three-dimensional descriptions. To describe the level of complexity for such descriptors, different dimensionalities (levels of complexity) are used, the descriptors being labelled as 1D, 2D, 3D, and 4D [23]. While it is reasonable to assume that 3D models would provide substantially more detail regarding a compound's activity or property, in practice, such models are typically restricted to relatively small series of similar compounds, in order to eliminate conformational uncertainty. On the other hand, 2D molecule representations are commonly used for large datasets. Furthermore, the molecular graphs provided by 2D representations are also useful for interpreting QSAR models via the use of chemical structure information (molecule fragments) [24].

Rajathei et al. (2020) developed a 2D-QSAR model for HMG-CoA reductase inhibitors, but it was based on only 30 pyrrole derivatives of atorvastatin [25]. Moorthy et al. (2015) developed an interesting set of QSAR models, based on both linear regression and classification, using MOE for the calculation of the molecular descriptors (2D and 3D). However, these authors did not report on using the models for virtual screening purposes and their validation was based on the techniques of leave one out (LOO), leave many out (LMO), and bootstrapping (besides randomization and holdout testing) [26]. Nested cross-validation, which is apt to provide a more reliable estimation of model performance and a better control of overfitting was not used in this interesting paper. Moreover, it is not clear from that paper whether the HMG-CoA reductase inhibitors were evaluated on a human or rodent version of the enzyme. Samizo and Kaneko (2023) developed QSAR models using a data set of 833 compounds from the ChEMBL database, but they used a HMG-CoA reductase of rat origin, not of human origin [27]. Zang et al. (2017) built a 3D-QSAR model based on a small sample size of 19 compounds, but targeting not human, but lepidopteran HMG-CoA reductase [28]. Another QSAR model was also built on a small number (n=18) of phthalimide congeners [29]. We report in this paper on a series of QSAR models developed for human HMG-CoA reductase inhibitors, using nested cross-validation as the main validation approach, and using the best performing-models for the virtual screening of over 220,000 chemical compounds from the ZINC 15 database. As a practical application of the models, we have also used them to understand what are the natural compounds responsible for the reported LDL-cholesterol lowering effect of an *Iris*  ×  *germanica* L. extract [30].

**2. Results**

2.1. Chemical space distribution and diversity of the compounds in the training data set

The variation of ALogP (a measure of lipophilicity, and indirectly, of membrane permeability [31]) as a function of the molecular weight is represented graphically in Fig. 1. The largest density was observed for molecular weights varying between 250 and 500 g mol−1 (first and third quartiles corresponded to 257.2 and 455.5, respectively), and for ALogP varying between 1 and 4. For active compounds (defined as having an IC50 < 100 nM), the minimum molecular weight in the data set was 369.4, the maximum 778.1, and the median value was 491.1 g mol−1. ALogP varied between 1.4 and 8.4 for the active compounds, with a median value of 4.9. We compared these data with those for ten statins that were at least partially developed as medicinal products (lovastatin, cerivastatin, atorvastatin, fluvastatin, simvastatin, rosuvastatin, glenvastatin, pravastatin, mevastatin, and pitavastatin) and found that for the latter molecular weight varied between 390.5 and 558.6, with a median value of 422.5 g mol−1. For statins, ALogP ranged between 2.1 and 5.5, with a median value of 4.2.

For the entire data set of HMG-CoA reductase inhibitors (all pairs), the average of the Tanimoto similarity coefficients was 0.59, and the first and third quartiles were 0.52 and 0.59 (Fig. 2). For the compounds forming the training, the average of the Tanimoto coefficients was also 0.53, whereas for the testing set, it was slightly higher, 0.59. The Tanimoto coefficients for the whole data set and the training and test subsets indicate a reasonably large chemical diversity for the compounds used in the modeling.



**Figure 1.** Chemical diversity representation of the HMGCoA-inhibitors data set (chemical space defined by the molecular weight (MW) and AK Ghose – G.M. Crippen logP (ALogP).



**Figure 2.** Chemical diversity representation of the HMGCoA-inhibitors data set - histogram of the Tanimoto similarity (based on MACCS fingerprints) for all possible unique pairs of chemical compounds from the data set.

Table 1 displays the number of failures of Lipinski's rule of five for both active and inactive compounds (defined as previously mentioned). One-third of the active compounds had no failure, whereas 19.56% had one failure, 22.46% two failures, and 24.64% three failures. No active compound had four or five failures of Lipinski’s rule. Interestingly, most inactive compounds (71.79%) had no Lipinsky failures.

Table 1. Number of failures of the Lipinski's rule of five for the compounds in the data set

|  |  |  |
| --- | --- | --- |
| **No. of failures** | **Active compounds** | **Inactive compounds** |
| 0 | 46 | 649 |
| 1 | 27 | 127 |
| 2 | 31 | 52 |
| 3 | 34 | 34 |
| 4 | 0 | 40 |
| 5 | 0 | 2 |

2.1. Regression models and their performance

We built and evaluated a number of 300 models through nested-cross validation (using different machine learning regression algorithms, feature selection methods, and fingerprint or descriptor data sets) (Tables S1-S6). From these, we selected a number of 21 models that performed reasonably well in the nested cross-validation (either R2 ≥ 0.70 or CCC ≥ 0.85, Table 2). Among the latter, only six met performance conditions (R2 ≥ 0.70 and CCC ≥ 0.85), and these were selected to also build five ensemble models. To do this, we used the predicted values from the external loop of the nested cross-validation results (using as the random seed the one that gave CCC values closest to the mean value of the five seeds tested; for example, for model no. 4, we used the predicted values for the seed that gave a CCC value of 0.853, as this was the closest to the mean value of 0.851 for that model).

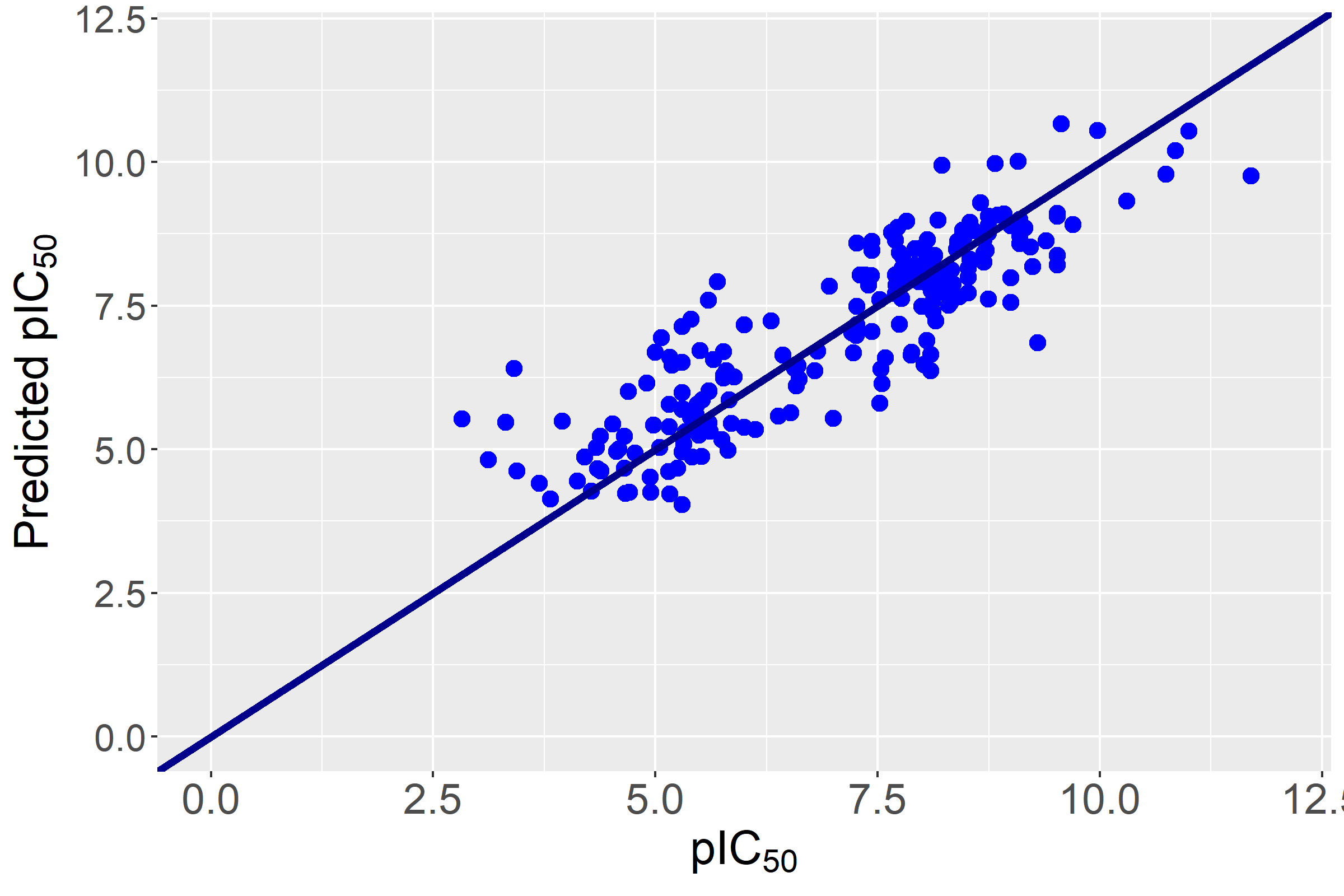
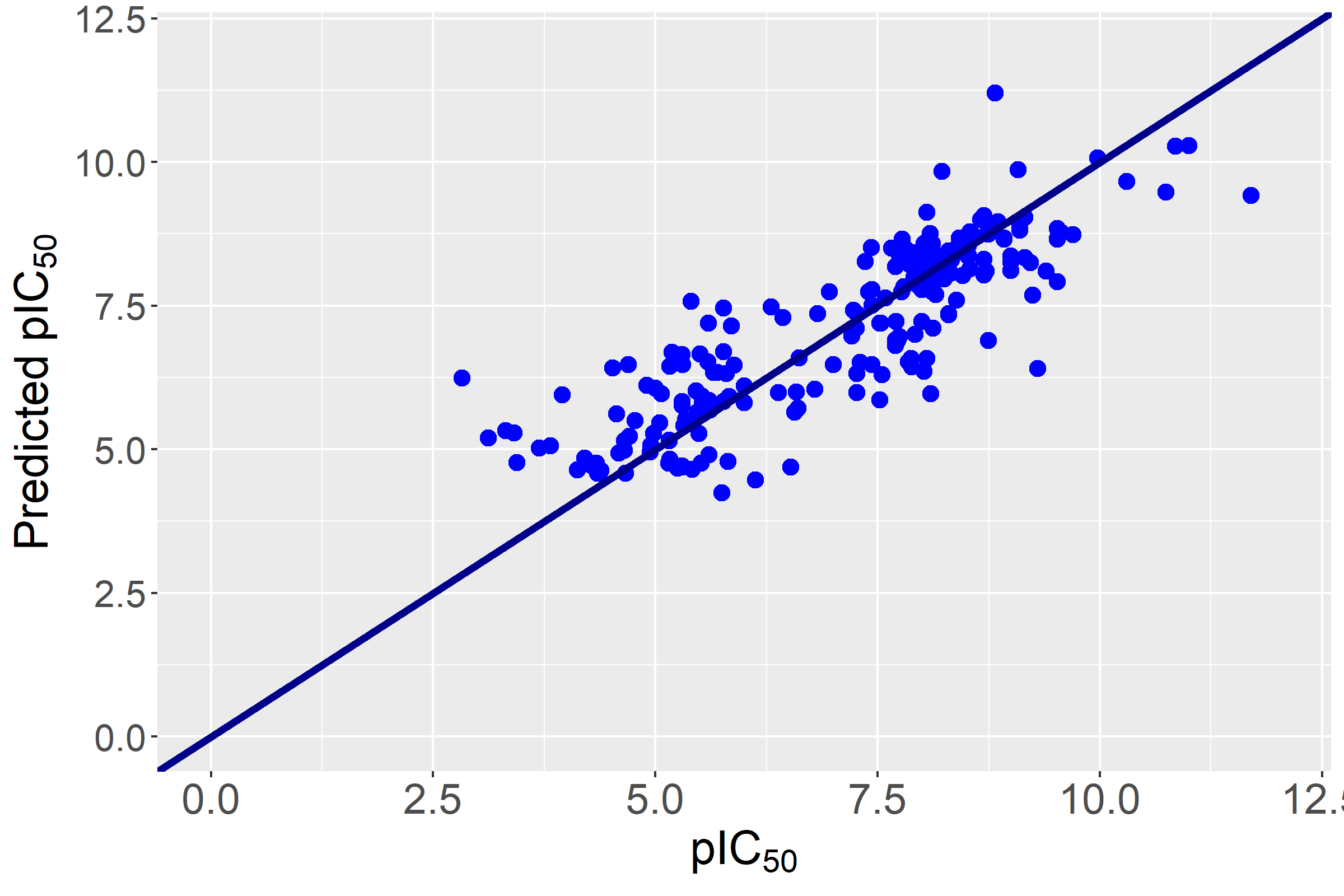
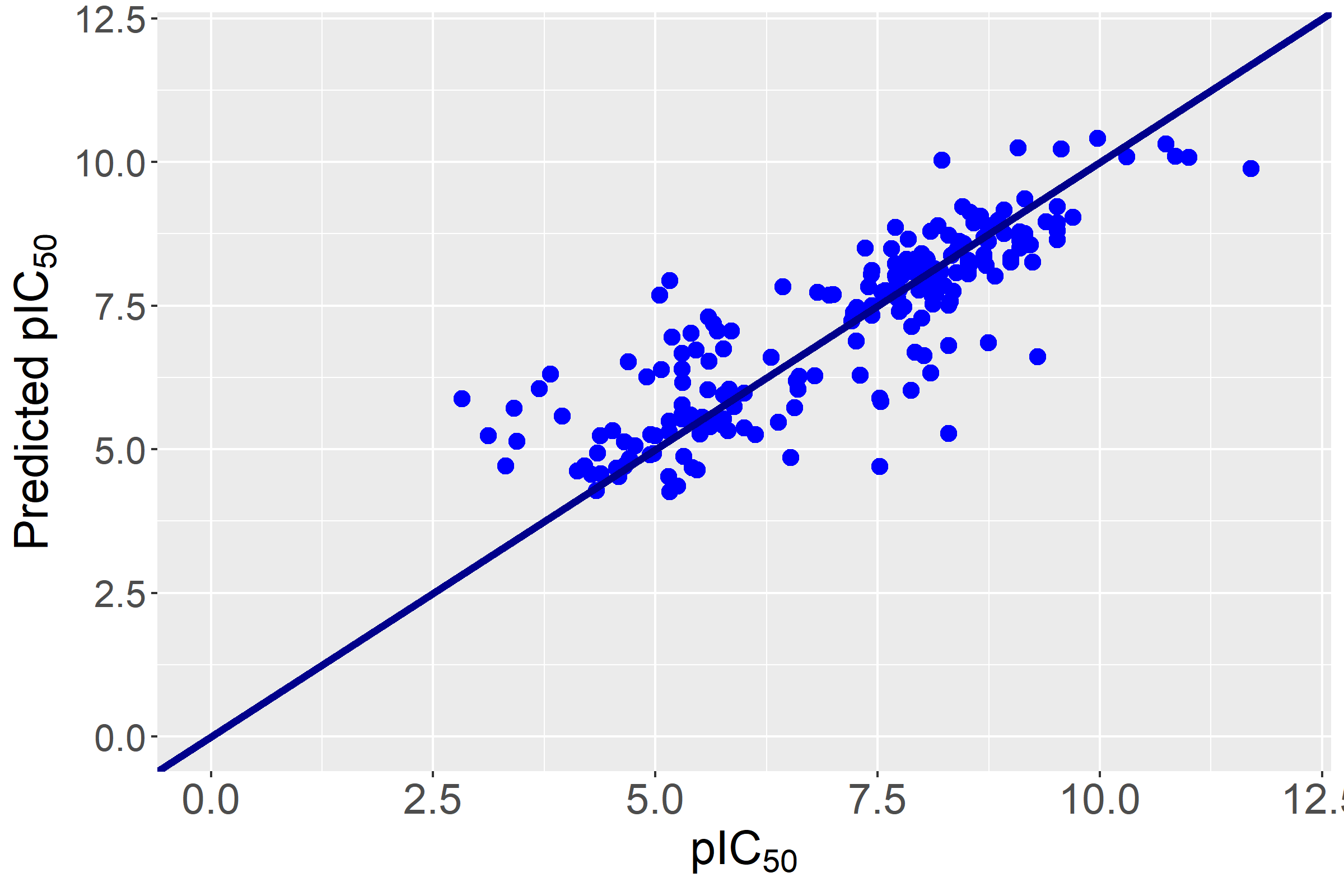
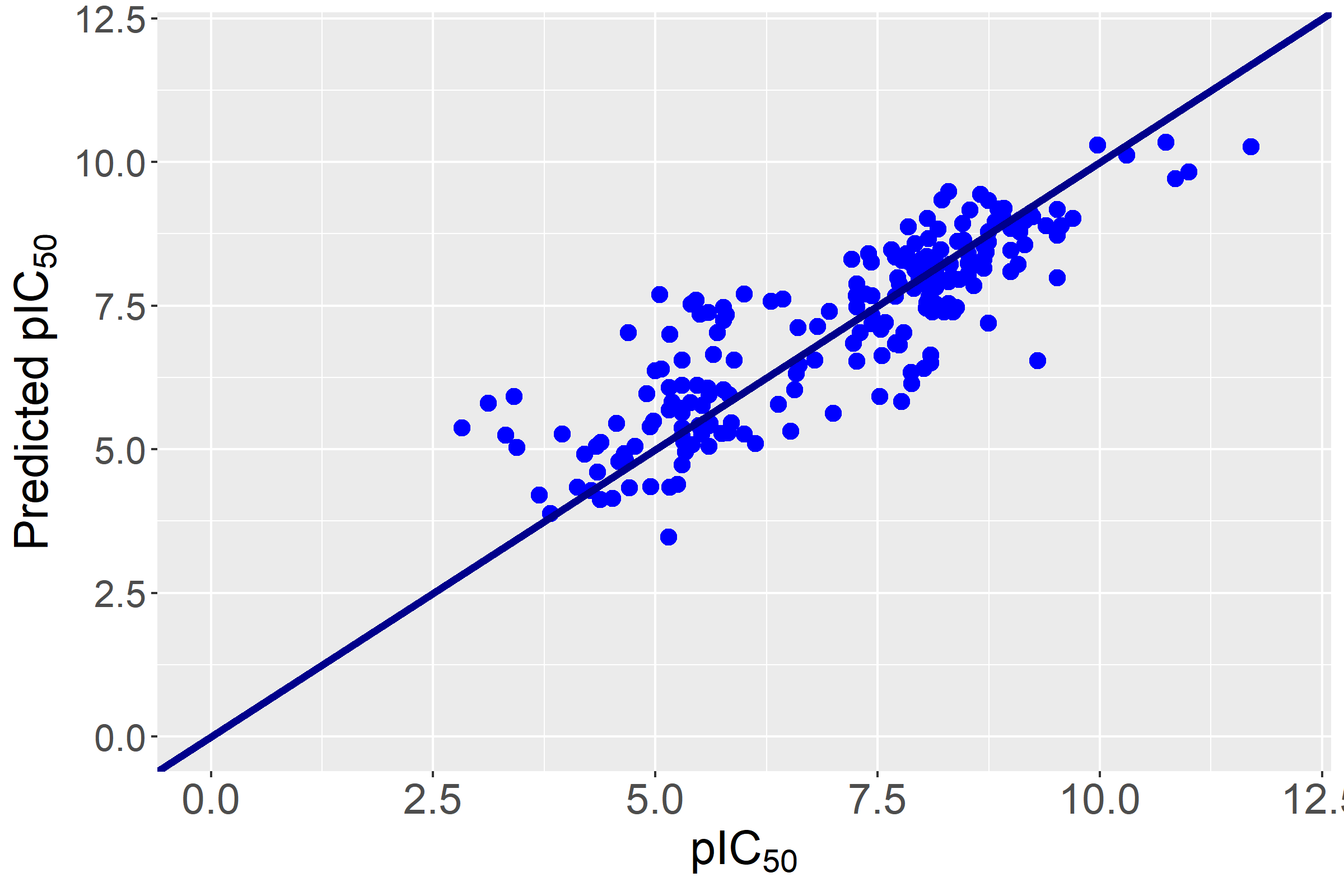
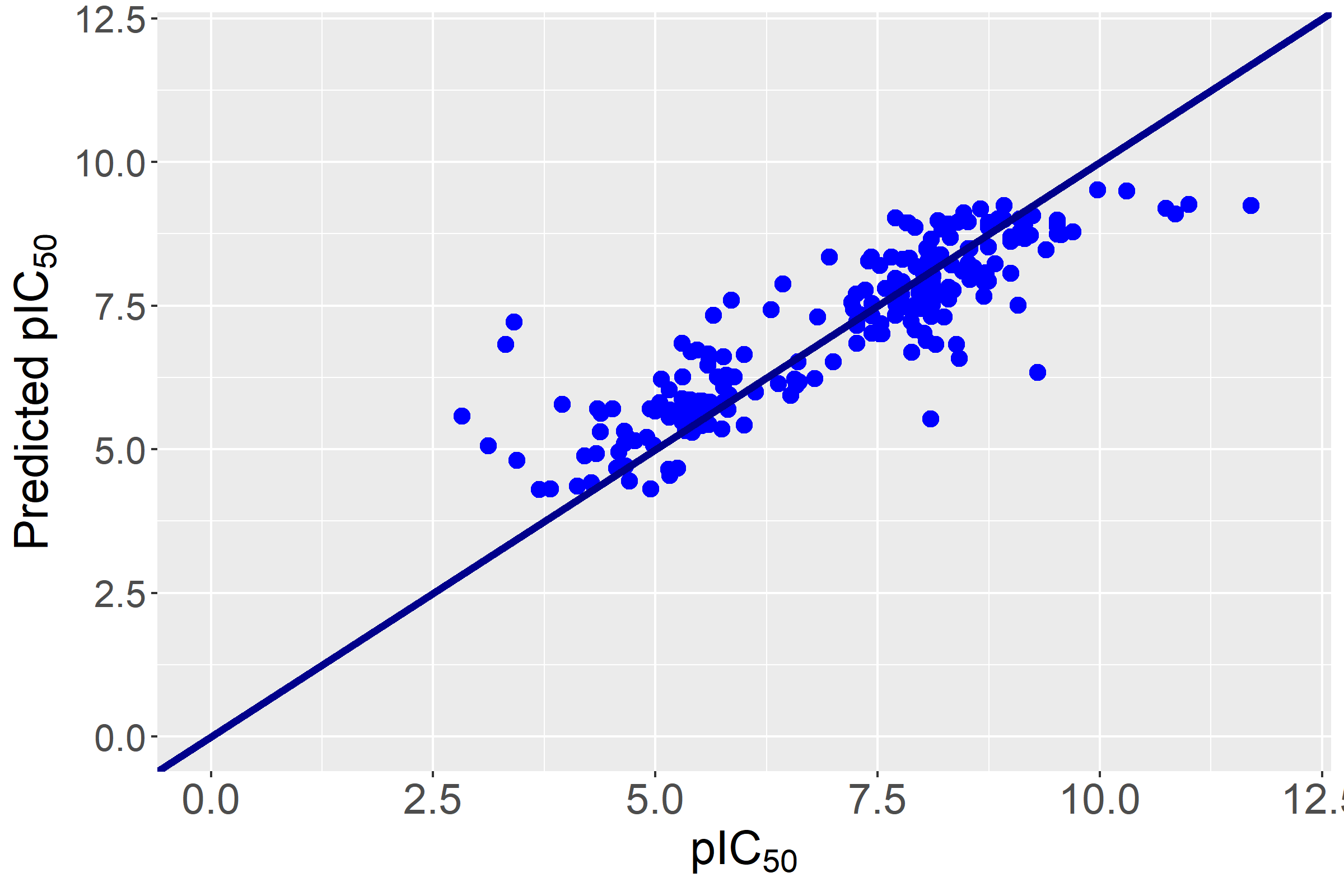
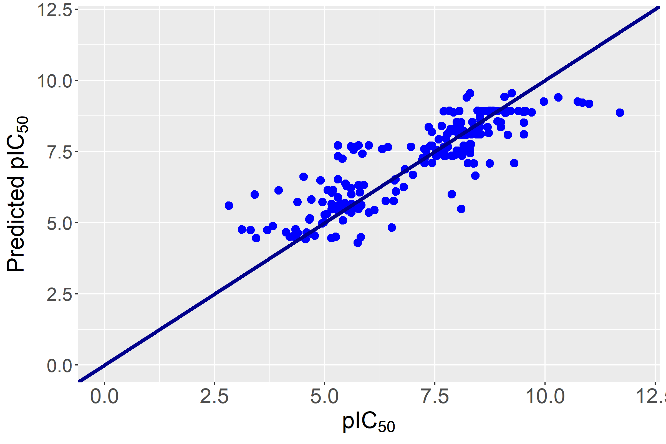
Table 2. Results of nested-cross validation for models with reasonably good performance. Five replicates were used with different random seeds.

| **No.** | **Regression algorithm** | **Descriptor set** | **Feature selection method** | **CCC (nested CV, n = 5)**  **mean (s.d.)** | **R2**  **(nested CV, n=5)**  **mean (s.d.)** | **RMSE**  **(n=5)**  **mean (s.d.)** |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | Random forest (“ranger”) | MACCS | “cmim” | 0.837  0.840  0.846  0.833  0.825  **0.836 (0.008)** | 0.707  0.716  0.745  0.698  0.720  **0.717 (0.018)** | 0.872  0.867  0.835  0.884  0.877  **0.867 (0.019)** |
| 2 | XGboost | MACCS | Boruta | 0.848  0.861  0.840  0.850  0.848  **0.849 (0.008)** | 0.726  0.744  0.725  0.701  0.741  **0.727 (0.017)** | 0.848  0.821  0.873  0.870  0.835  **0.849 (0.022)** |
| 3 | Random forest (“ranger”) | MACCS | Boruta | 0.835  0.827  0.833  0.826  0.834  **0.831 (0.004)** | 0.712  0.696  0.722  0.679  0.707  **0.702 (0.016)** | 0.890  0.903  0.877  0.916  0.886  **0.891** **(0.018)** |
| 4 | Support vector machines | MACCS | Boruta | 0.857  0.853  0.857  0.843  0.845  **0.851 (0.007)** | 0.743  0.740  0.754  0.708  0.738  **0.737 (0.017)** | 0.832  0.831  0.815  0.872  0.839  **0.838 (0.021)** |
| 5 | Gradient boosting machine (“GBM”) | Set2 | Boruta | 0.858  0.820  0.827  0.830  0.829  **0.833 (0.015)** | 0.752  0.681  0.702  0.696  0.667  **0.700 (0.032)** | 0.815  0.942  0.912  0.915  0.926  **0.902 (0.050)** |
| 6 | Support vector machines | Set2 | “jmim” | 0.840  0.841  0.850  0.839  0.841  **0.842 (0.004)** | 0.734  0.727  0.756  0.728  0.746  **0.738 (0.012)** | 0.854  0.850  0.824  0.849  0.838  **0.843 (0.012)** |
| 7 | BART | Set2 | Gaselect | 0.846  0.848  0.854  0.850  0.845  **0.849 (0.004)** | 0.730  0.739  0.745  0.733  0.689  **0.727 (0.022)** | 0.858  0.833  0.830  0.847  0.864  **0.846 (0.015)** |
| 8 | Random forest (“ranger”) | Set2 | Gaselect | 0.827  0.830  0.823  0.830  0.818  **0.826 (0.005)** | 0.733  0.730  0.708  0.742  0.723  **0.727 (0.013)** | 0.848  0.849  0.864  0.850  0.874  **0.857 (0.011)** |
| 9 | XGboost | Set2 | “jmim” | 0.832  0.843  0.832  0.830  0.823  **0.832 (0.007)** | 0.724  0.724  0.706  0.684  0.705  **0.709 (0.017)** | 0.868  0.852  0.885  0.890  0.901  **0.879 (0.019)** |
| 10 | BART | Set2 | Boruta | 0.867  0.825  0.825  0.834  0.829  **0.836 (0.018)** | 0.764  0.690  0.697  0.707  0.674  **0.704 (0.034)** | 0.797  0.929  0.917  0.901  0.919  **0.893 (0.054)** |
| 11 | Rule- and instance-cased regression | Set2 | Gaselect | 0.837  0.821  0.835  0.843  0.823  **0.832 (0.009)** | 0.724  0.707  0.717  0.727  0.660  **0.707 (0.027)** | 0.860  0.891  0.881  0.851  0.893  **0.875 (0.019)** |
| 12 | Support vector machines | Set2 | Gaselect | 0.849  0.853  0.840  0.856  0.851  **0.850 (0.006)** | 0.748  0.754  0.715  0.766  0.757  **0.748 (0.020)** | 0.821  0.804  0.846  0.804  0.819  **0.819 (0.017)** |
| 13 | Random forest (“ranger”) | Set3 | Gaselect | 0.812  0.832  0.826  0.826  0.823  **0.824 (0.007)** | 0.702  0.720  0.727  0.731  0.717  **0.719 (0.011)** | 0.873  0.841  0.860  0.862  0.876  **0.862 (0.014)** |
| 14 | BART | Set4 | “jmim” | 0.864  0.845  0.858  0.853  0.852  **0.854 (0.007)** | 0.751  0.710  0.742  0.731  0.730  **0.733 (0.015)** | 0.821  0.888  0.844  0.858  0.856  **0.853 (0.024)** |
| 15 | Weighted k-Nearest Neighbor | Set4 | Boruta | 0.826  0.854  0.865  0.848  0.858  **0.850 (0.015)** | 0.690  0.740  0.739  0.709  0.737  **0.723 (0.022)** | 0.923  0.846  0.821  0.874  0.858  **0.864 (0.038)** |
| 16 | BART | Set4 | Gaselect | 0.856  0.847  0.845  0.846  0.859  **0.851 (0.006)** | 0.743  0.726  0.715  0.719  0.745  **0.730 (0.014)** | 0.833  0.865  0.871  0.877  0.848  **0.859 (0.018)** |
| 17 | XGboost | Set4 | “jmim” | 0.835  0.832  0.856  0.830  0.846  **0.840 (0.011)** | 0.701  0.702  0.750  0.705  0.737  **0.719 (0.022)** | 0.857  0.896  0.825  0.902  0.844  **0.865 (0.033)** |
| 18 | Random forest (“ranger”) | Set4 | Boruta | 0.831  0.846  0.857  0.855  0.847  **0.847 (0.010)** | 0.702  0.748  0.754  0.749  0.738  **0.738 (0.021)** | 0.861  0.835  0.796  0.829  0.848  **0.834 (0.024)** |
| 19 | Rule- and instance-cased regression | Set4 | Gaselect | 0.851  0.813  0.841  0.842  0.837  **0.837 (0.014)** | 0.726  0.655  0.721  0.715  0.688  **0.701 (0.030)** | 0.859  0.947  0.882  0.879  0.896  **0.893 (0.033)** |
| 20 | BART | Set4 | Boruta | 0.856  0.870  0.868  0.872  0.877  **0.869 (0.008)** | 0.743  0.757  0.743  0.760  0.768  **0.754 (0.011)** | 0.833  0.814  0.836  0.805  0.800  **0.818 (0.016)** |
| 21 | XGboost | Set4 | Boruta | 0.850  0.849  0.850  0.855  0.843  **0.849 (0.004)** | 0.737  0.747  0.734  0.738  0.711  **0.733 (0.013)** | 0.848  0.834  0.838  0.842  0.893  **0.851 (0.024)** |

The 21 models with reasonably good performance were based on seven different algorithms: random forests (five models), BART (five models), boosting algorithms (Xgboost – four models and GBM – one model), support vector machines (three models), rule- and instance-based regression (two models) and weighted k-nearest neighbor (one model). However, the six best performing models were built with the following algorithms: support vector machines (two models), BART (three models) and weighted k-nearest neighbor (one model). With respect to feature selection algorithms, among the six best performing models three were built with the help of Boruta, two with “gaselect” and one with the “jmim” algorithm. Among the 21 selected models, nine were built with Boruta, seven with “gaselect”, four with “jmim”, and one with “cmim”.

Table 3. Performance of the five ensemble models

|  |  |  |  |
| --- | --- | --- | --- |
| **Ensemble algorithm** | **CCC (nested CV)** | **R2 (nested cross-validation)** | **RMSE (nested cross-validation)** |
| Support vector machines | 0.893 | 0.798 | 0.730 |
| BART | 0.888 | 0.789 | 0.745 |
| KKNN | 0.887 | 0.789 | 0.750 |
| Random forests | 0.889 | 0.794 | 0.739 |
| Xgboost | 0.883 | 0.784 | 0.760 |



**C**

**D**

**A**

**B**

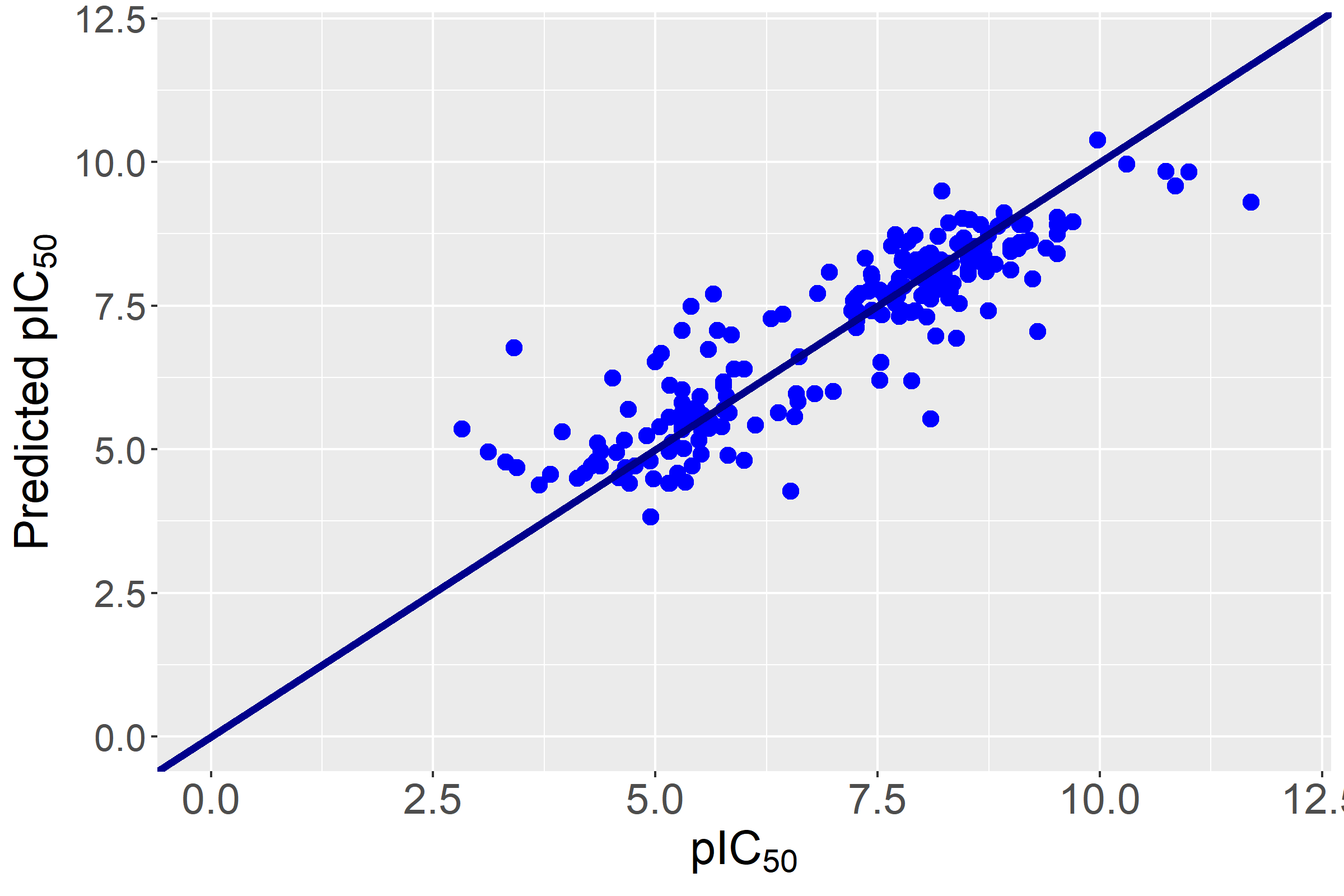
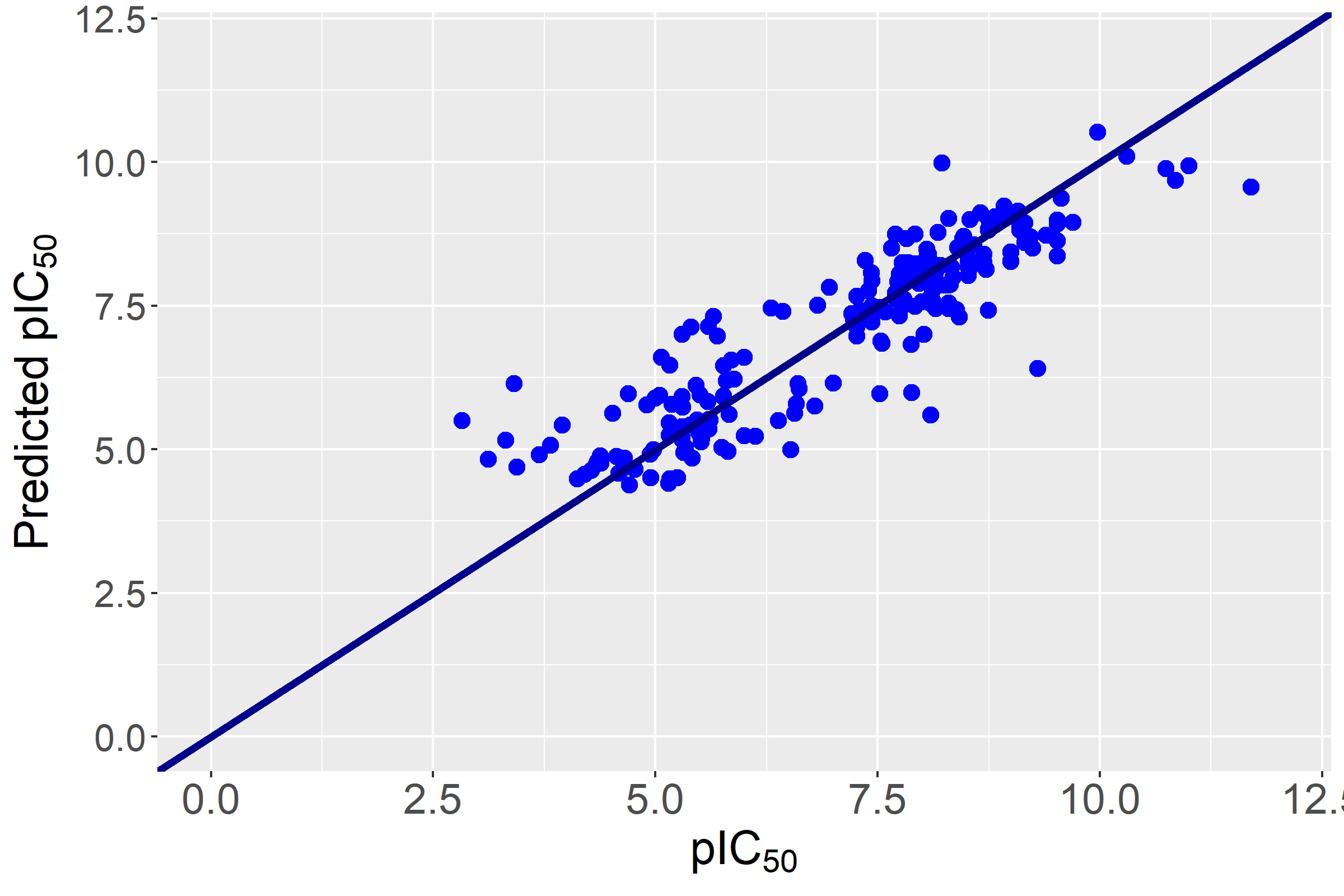
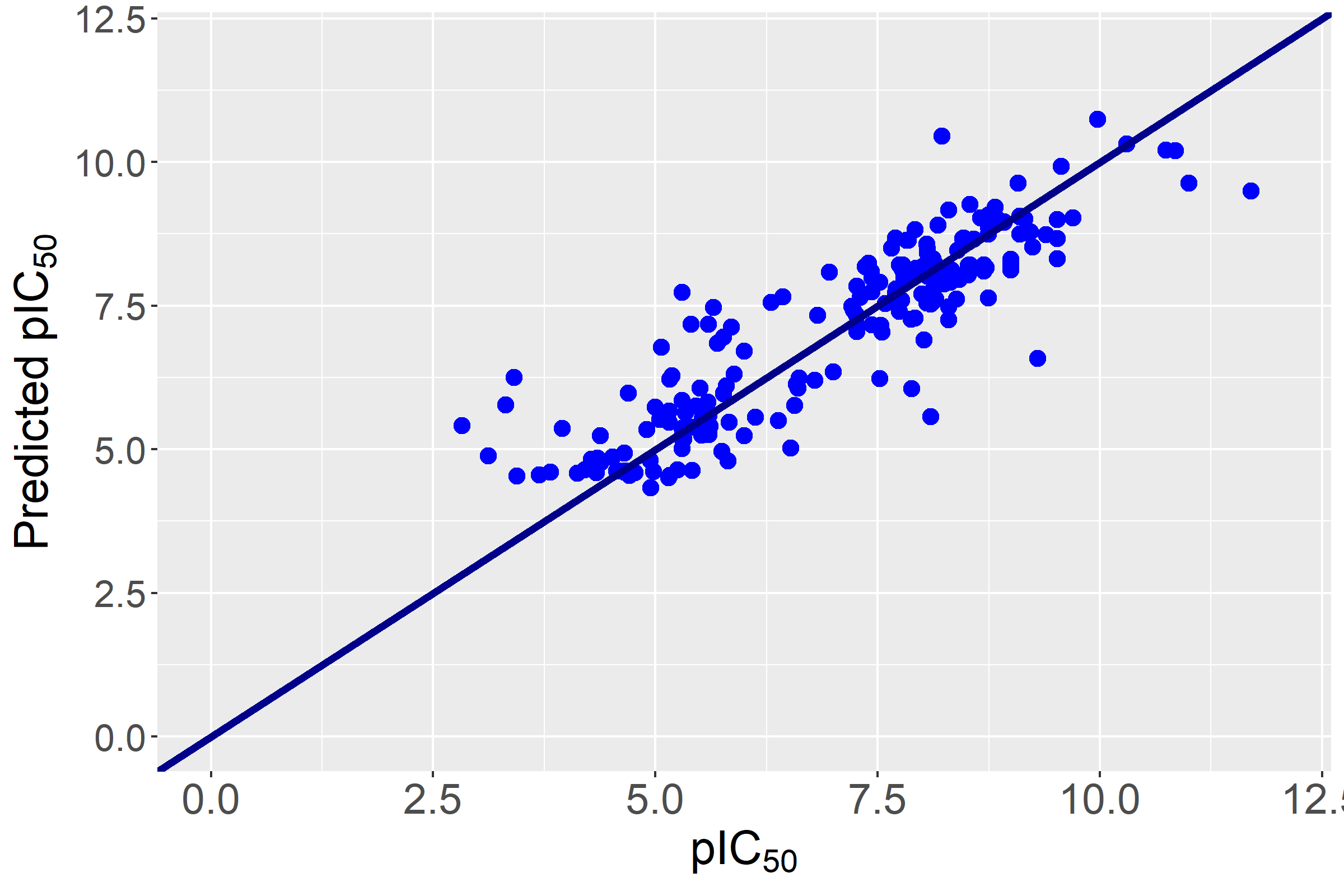
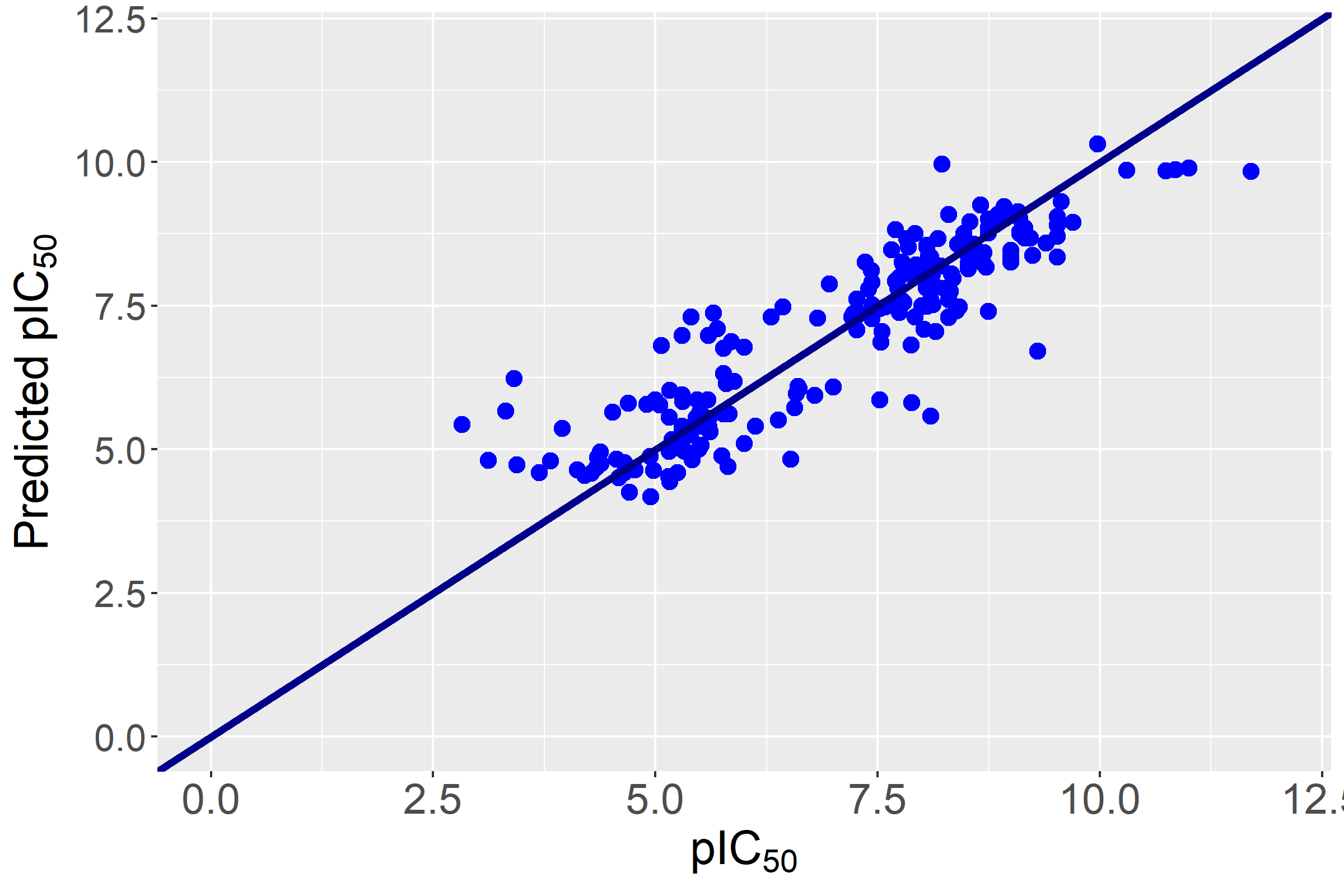
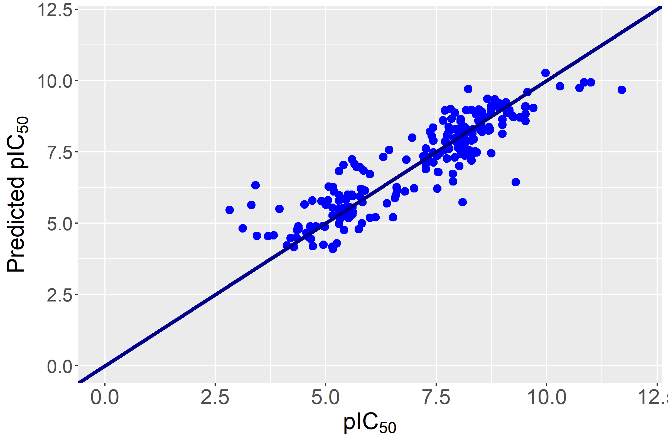
**E**

**F**

Figure 3. Experimental vs. predicted pIC50 for the six best-performing regression models (which were selected to build the ensemble models).

**Figure 3**. Experimental vs. predicted pIC50 for the six best-performing regression models (which were selected to build the ensemble models). The sloping line represents perfect agreement between actual and predicted values. Points above this line indicate overpredictions, while points below indicate underpredictions.

Five ensemble models were built, each using the predicted values in the external loop of the six best performing models (models no. 4, 12, 14-16, and 20 in Table 2) and five different tree-based algorithms: support vector machines, BART, weighted k-nearest neighbor, random forests, and XGboost. The performance of these ensemble models is show synthetically in Table 3.



**A**

**B**

**C**

**E**

**D**

**Figure 4**. Experimental vs. predicted pIC50 for the five ensemble models. A. SVM. B. BART. C. KKNN. D. Random forests. E. Xgboost. The sloping line represents perfect agreement between actual and predicted values. Points above this line indicate overpredictions, while points below indicate underpredictions.

y-randomization

We permuted the response variable in the initial data set and thereafter followed the same procedure of feature selection and nested cross-validation as for the authentic data set and computed the same performance metrics (Table 4). Whereas in the feature selection with simple cross-validation a few of the models had reasonable performance, in the nested cross-validation all three metrics indicated overwhelming underperformance. This provides evidence that the relationship underlying the models selected is genuine and the good performance is not the mere chance result of good machine learning algorithms.

Table 4. Performance metrics for three representative data sets and algorithms for which the response variable was permuted 20 times

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model whose features were randomized | **CCC (nested CV, n = 20)**  **mean (s.d.)** | **Rr2**  **(nested CV, n=20)**  **mean (s.d.)** | **RMSE**  **(n=20)**  **mean (s.d.)** | **Rp2 (for the corresponding model)** |
| Model 19 in Table 2 | 0.047 (0.055) | -0.220 (0.077) | 1.804 (0.045) | 0.803 |
| Model 17 in Table 2 | -0.007 (0.034) | -0.113 (0.068) | 1.731 (0.027) | 0.773 |
| Model 20 in Table 2 | 0.078 (0.060) | -0.056 (0.040) | 1.685 (0.032) | 0.781 |

In the literature it has been proposed that an R2p value should be computed as , where R2 is the value of the non-random model and the , the mean R2 value of the randomized models. An ideal QSAR model should have close to zero and and close to R2 for the genuine model [32]. The y-randomization tests and the values have convincingly confirmed that the models selected are not the result of mere chance.

Descriptors useful for HMGCo-A inhibition prediction

Using DALEX and iml R packages, we analyzed the descriptors with the highest importance in explaining the HMGCoA inhibition in the six best-performing models.

The most important MACCS keys identified in the best-performing model used with this type of descriptors (model no. 4 in Table 2), along with their structural significance and impact on activity are shown in Table 5 and Figure S1 and S2.

Table 5. Important MACCS keys identified in model no. 4 (from Table 2), their corresponding structural patterns and their association with the HMGCoA inhibitory activity

| MACCS Key | Structural Pattern | Association |
| --- | --- | --- |
| 62 | "A$A!A$A" (any atom – ring bond – any atom – chain bond – any atom – ring bond – any atom) | Positive |
| 85 | CN(C)C (a closed ring formed by a C-N-C chain) | Positive |
| 105 | "A$A($A)$A" (aromatic atom – substructure – aromatic atom) | Negative |
| 22 | Three-membered ring system (3M ring) | Relatively strongly negative |
| 65 | Carbon and nitrogen united by an aromatic query bond | Positive |
| 145 | 6M RING > 1 (more than one six-member rings) | Positive |
| 89 | OAAAO (two oxygen atoms connected by three other atoms) | Positive |
| 97 | NAAAO (a nitrogen atom connected by a sequence of four single bonds to an oxygen atom) | Weakly negative |
| 107 | XA(A)A (where X is a halogen and A any atom) | Weakly positive |
| 42 | F (a fluorine atom) | Weakly positive |

For the model based on the second set of descriptors (2D matrix-based descriptors, 2D autocorrelations, and Burden eigenvalues) (model no. 12 in Table 2), the most strongly associated with the HMGcoA reductase inhibition are summarized in Table 6 and Figures S3 and S4. For each key descriptor in the model, we have also provided the descriptors highly correlated with it, because one important descriptor could just stand as a proxy for other highly correlated descriptor or, alternatively, may be more intuitively understood in this way and thus facilitate the interpretation of its contribution. The activity relationship is described based on the partial dependence plots; although such plots are useful in understanding the way a feature associates with the response variable, they may not capture the full complexity of interactions between the contributing features [33].

Table 6. Key Descriptors Utilized in the Regression Model Constructed using the Set 2 descriptors (2D matrix-based descriptors, 2D autocorrelations, and Burden eigenvalues) (model no. 12 in Table 2). The model employed SVM as the regression algorithm with the genetic algorithm (“gaselect”) as a feature selection method.

| Descriptor | Correlation coefficient (for other descriptors) | Correlated Descriptors | Activity Relationship |
| --- | --- | --- | --- |
| MATS3e  (Moran autocorrelation of lag 3 weighted by Sanderson electronegativity) | r = 0.846 | MATS3s (Moran autocorrelation of lag 3 weighted by I-state) | Negative values → higher activity |
| SpMax\_B(p)  (Leading eigenvalue from Burden matrix weighted by polarizability) | r >0.91  r>0.80 | SpDiam\_B(p) (Diameter from Burden matrix weighted by polarizability)  SpMax1\_Bh(p) (Leading eigenvalue n. 1 of Burden matrix weighted by polarizability)  piPC06 (molecular multiple path count of order 6)  SpDiam\_B(v) ( spectral diameter from Burden matrix weighted by van der Waals volume)  SpMax\_B.v. | Inverted U-shape |
| VE1sign\_B(s)  (Coefficient sum of the last eigenvector from Burden matrix weighted by I-State) | N/A | None | Higher values → lower activity |
| SpMin1\_Bh(e)  (Smallest eigenvalue n. 1 of Burden matrix weighted by Sanderson electronegativity) | r = 0.99  r>0.87  -0.80 | SpMin1\_Bh(i) (Smallest eigenvalue n. 1 of Burden matrix weighted by ionization potential)  SpMin1\_Bh(v) (Smallest eigenvalue n. 1 of Burden matrix weighted by van der Waals volume)  SpMin1\_Bh(p) (Smallest eigenvalue n. 1 of Burden matrix weighted by polarizability)  WiA\_D/Dt (average Wiener-like index from distance/detour matrix) | Negative association with an asymmetric inverted U-shape |
| SM3\_X  (Spectral moment of order 3 from chi matrix) | r > 0.90  r=0.81 | nR03 (Number of 3-membered rings)  D/Dtr03 (Distance/detour ring index of order 3)  SRW03 (Self-returning walk count of order 3)  SM5\_X (Spectral moment of order 5 from chi matrix)  B04[N-S] (Presence/absence of N – S at topological distance 4)  B06[O-S] (Presence/absence of O – S at topological distance 6)  F06[O-S] (Frequency of O – S at topological distance 6) | Negative correlation with pIC50 |
| GATS5v  (Geary autocorrelation of lag 5 weighted by van der Waals volume) | r = -0.903  r = 0.80 | MATS5p (Moran autocorrelation of lag 5 weighted by polarizability)  GATS5p (Geary autocorrelation of lag 5 weighted by polarizability) | Increasing values → higher activity |
| MATS1p  (Moran autocorrelation of lag 1 weighted by polarizability) | r = 0.93  r = 0.87 | MATS1v (Moran autocorrelation of lag 1 weighted by van der Waals volume), MATS1i (Moran autocorrelation of lag 1 weighted by ionization potential) | Inverted U-shaped relationship with activity |
| JGI5  (Mean topological charge index of order 5) | NA | None | Higher values → higher inhibitory activity |
| TI2\_L  (Second Mohar index from Laplace matrix) | r > 0.8 for all but none > 0.9 | MSD (Mean square distance index (Balaban))  AECC (Average eccentricity)  DECC (Eccentric)  ICR (Radial centric information index)  MaxTD (Max topological distance)  S3K (3-path Kier alpha-modified shape index)  IDE (Mean information content on the distance equality)  HVcpx (Graph vertex complexity index)  WiA\_Dz(Z) (Average Wiener-like index from Barysz matrix weighted by atomic number)  SpPosA\_Dz(Z) (Normalized spectral positive sum from Barysz matrix weighted by atomic number)  SpMaxA\_Dz(Z) (Normalized leading eigenvalue from Barysz matrix weighted by atomic number)  SpMAD\_Dz(Z) (Spectral mean absolute deviation from Barysz matrix weighted by atomic number)  WiA\_Dz(m) (Average Wiener-like index from Barysz matrix weighted by mass)  SpPosA\_Dz(m) (Normalized spectral positive sum from Barysz matrix weighted by mass)  SpMaxA\_Dz(m) (Normalized leading eigenvalue from Barysz matrix weighted by mass)  SpMAD\_Dz(m) (Spectral mean absolute deviation from Barysz matrix weighted by mass)  WiA\_Dz(v) (Average Wiener-like index from Barysz matrix weighted by van der Waals volume)  SpPosA\_Dz(v) (Normalized spectral positive sum from Barysz matrix weighted by van der Waals volume)  SpMaxA\_Dz(v) (Normalized leading eigenvalue from Barysz matrix weighted by van der Waals volume)  SpMAD\_Dz(v) (Spectral mean absolute deviation from Barysz matrix weighted by van der Waals volume)  WiA\_Dz(e) (Average Wiener-like index from Barysz matrix weighted by Sand | Higher values → lower inhibitory activity |

For model no. 14, built with descriptors from the set 4 (functional group counts, atom-centred fragments, atom-type E-state indices, and pharmacophore descriptors), using BART as a regression algorithm and “jmim” as a feature selection method, the most important descriptors are summarized in Table 7 and Figures S5 and S6.

Table 7. Key Descriptors Utilized in the Regression Model Constructed using the Set 4 descriptors (functional group counts, atom-centered fragments, atom-type E-state indices, and pharmacophore descriptors) (model no. 14 in Table 2). The model employed BART as the regression algorithm with the 'jmim' as a feature selection method.

| **Descriptor** | **Correlated Descriptors** | **Correlation coefficient(s)** | **Activity Relationship** |
| --- | --- | --- | --- |
| C-034 (R–CR..X) | nPyrroles (number of pyrrole rings), N-073 (Ar2NH / Ar3N / Ar2N-Al / R..N..R), SaasN (sum of aasN E-states), NaasN (number of atoms of type aasN) | R=0.89 – 0.90 | Higher values → higher activity |
| SHED\_AA (Shannon entropy descriptor, acceptor-acceptor) | SHED\_DA (Shannon entropy descriptor, acceptor-acceptor) | r=0.91 | Lower values → higher activity |
| C-003 (a CHR3 group) | nCt (number of total tertiary C), nCrt (number of ring tertiary C) | r=0.88 - 0.99 | ≤3 → lower activity, 4 or 5 → higher activity |
| nCrt (number of ring tertiary C) | nCt, C-003, SpMin1\_Bh(s) (smallest eigenvalue n. 1 of Burden matrix weighted by I-state) | 0.80 – 0.88 | 0 → higher activity, ≥1 → lower activity |
| CATS2D\_04\_AA (CATS2D Acceptor-Acceptor at lag 04) | F04[O-O] (Frequency of O – O at topological distance 4) | r=0.81 | ≥3 → Stronger activity |
| NsF (number of atoms of type sF, i.e. -F) | nF (number of fluorine atoms), nX (number of halogen atoms), P\_VSA\_e\_6 (P\_VSA-like on Sanderson electronegativity, bin 6), F-084 (F attached to C1(sp2)), SsF (sum of sF E-states), NsF (number of atoms of type sF), F01[C-F] (frequency of C – F at topological distance 1), F02[C-F] (frequency of C – F at topological distance 2), F03[C-F] (frequency of C – F at topological distance 3), F07[C-F] (frequency of C – F at topological distance ), F08[C-F] (frequency of C – F at topological distance 8) | r>0.9 or r=1.0 | Fluorinated → higher activity |
| CATS2D\_04\_DA (CATS2D Donor-Acceptor at lag 04) | CATS2D\_04\_DD, F04[O-O] | r > 0.80 | Higher values → slightly higher inhibition |
| SHED\_AN (Shannon entropy descriptor, acceptor-negative) | SHED\_DN, CATS2D\_01\_DN (CATS2D Donor-Negative at lag 01), CATS2D\_00\_NN (CATS2D Negative-Negative at lag 00, i.e. number of negative atoms) | r>0.90 | Higher values → slightly lower activities |
| CATS2D\_02\_AL (CATS2D acceptor-lipophilic at lag 02) | F04[O-O] | r = 0.84 | Higher values → slightly higher inhibition |
| CATS2D\_09\_DL (CATS2D Donor-Lipophilic at lag 09) | CATS2D\_02\_DL, CATS2D\_07\_DL, CATS2D\_08\_DL | r > 0.80 | Lower values → higher inhibitory activity |

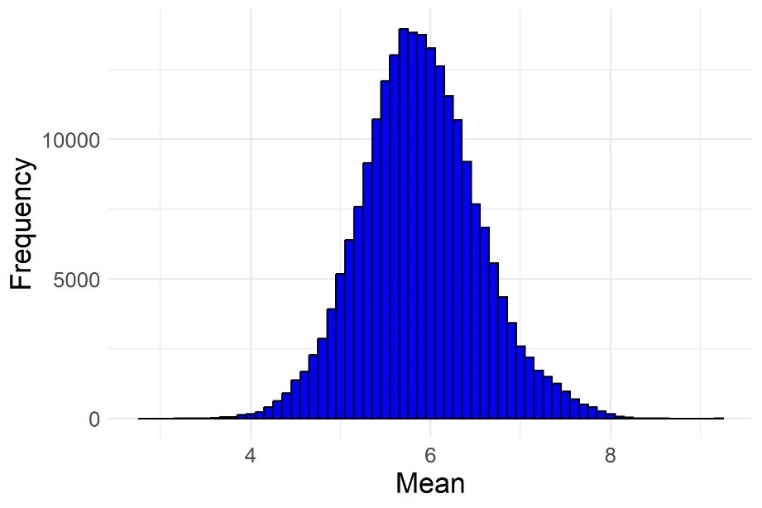
For the model no. 15, built with descriptors from the set 4 (functional group counts, atom-centred fragments, atom-type E-state indices, and pharmacophore descriptors), using KKNN as a regression algorithm and “Boruta” as a feature selection method, the most important descriptors are summarized in Table S7 and Figures S7 and S8.

For the model no. 16 (Table 2), built with descriptors from the set 4 (functional group counts, atom-centred fragments, atom-type E-state indices, and pharmacophore descriptors), using BART as a regression algorithm and “gaselect” as a feature selection method, the most important descriptors are summarized in Table S8 and Figures S9 and S10.

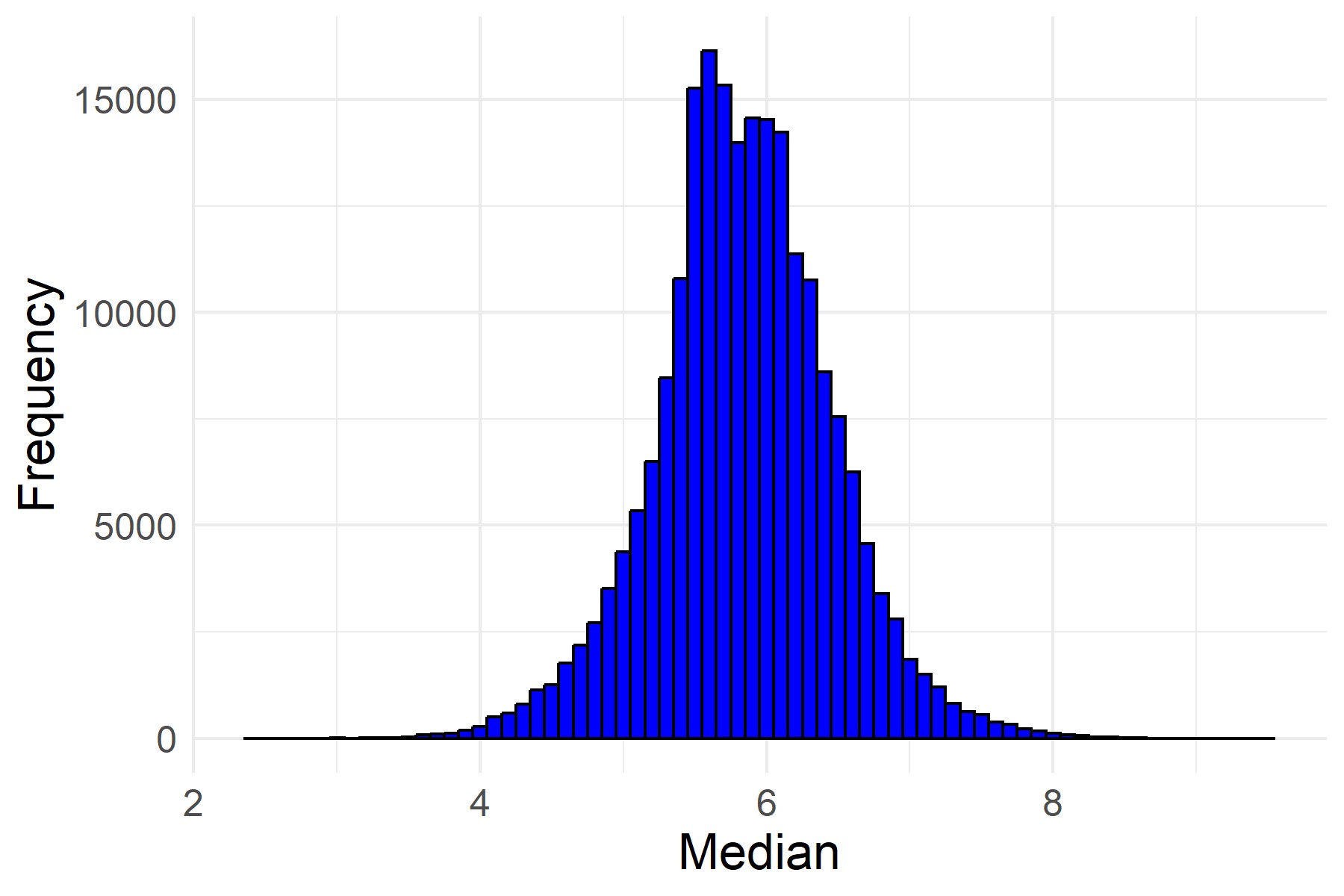
For the model no. 20, built with descriptors from the set 4 (functional group counts, atom-centred fragments, atom-type E-state indices, and pharmacophore descriptors), using BART as a regression algorithm and “Boruta” as a feature selection method, the most important descriptors are summarized in Table S9 and Figures S11 and S12.

Virtual screening of a data set of natural compounds

We have used the six best-performing models (and the best ensemble model (svm)) to virtually screen a number of almost 220,000 chemical compounds (mostly natural) from the ZINC 15 database [34]. The distribution of the mean predicted pIC50 values is shown in Figure 5. Only 237 compounds had a mean of pIC50 reliable predictions (i.e. inside the AD) equal to or higher than 8 and 287 had the median of reliable predictions higher than 8 (i.e. had IC50 values equal to or lower than 10 nM). Using the svm-based ensemble model, a number of 168 compounds (about 0.08%) had predicted IC50 values lower than 10 nM.



**Figure 5**. Histograms illustrating the distribution of the predicted mean (left) and median (right) pIC50 values for the 219,897 screened natural compounds from the ZINC 15 database



The distribution of the relative standard deviation (RSD, which expresses how well the predictions for each compound agrees among themselves) for the virtually screened compounds is shown in Figure 6. The mean and median RSD was around 13% (13.27% and 13.74%), the minimum RSD was 0.04%, whereas the maximum RSD was 63.16%. For most compounds the predictions were relatively close to one another, as for 75% of the predictions the RSD was less than 17.5%, i.e. there was at least moderate consistency for about three quarters of the data. However, this also means that for about one quarter of cases, despite selecting models with similar performance, the predictions differed to an important extent.

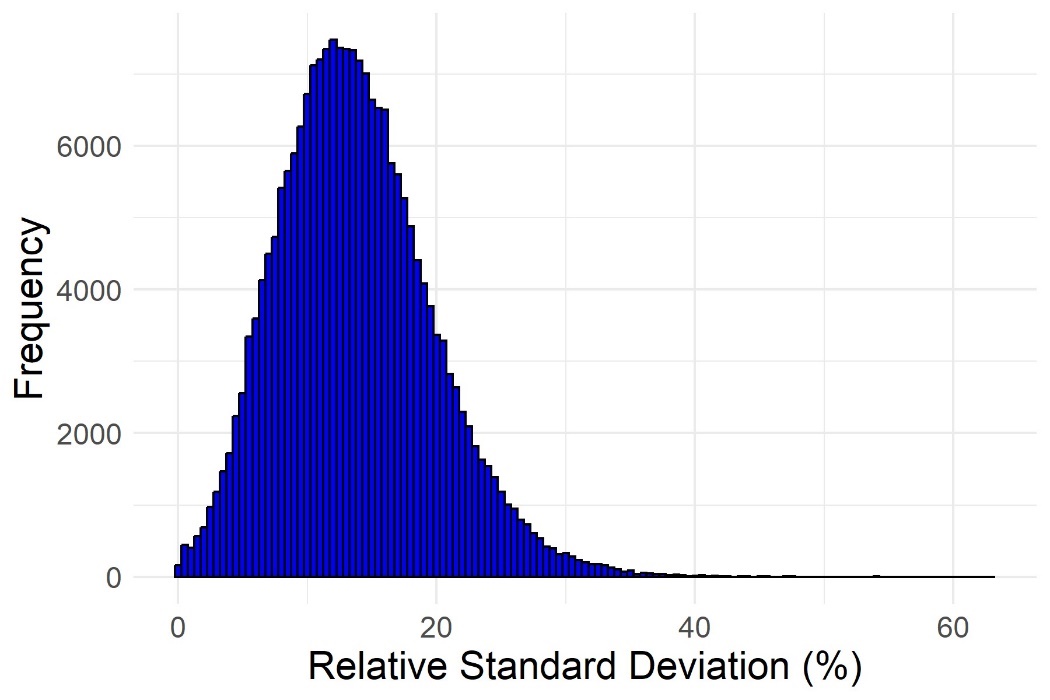
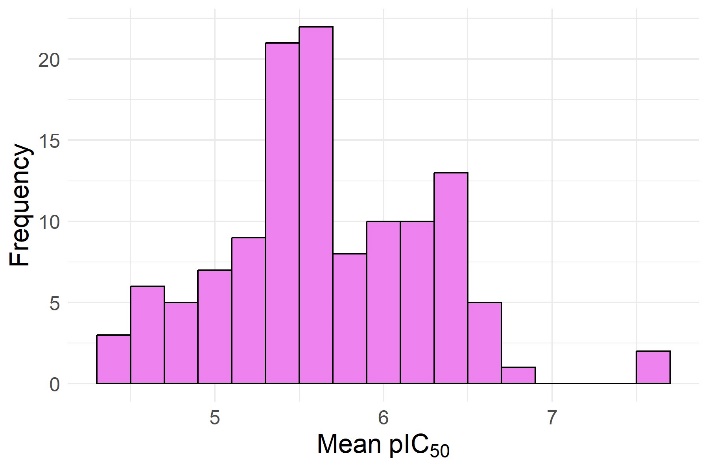


Figure 6. Distribution of the relative standard deviations (RSD) of the predictions made by the six selected models for each of the 219,897 virtually screened compounds.

A number of 81508 compounds (37.07% of all screened compounds) were inside the AD of all six models, and 88046 compounds (40.04%) were inside AD of five of the six models. At the opposite pole, 1758 compounds (0.80%) were outside the AD for all six models, 4550 (2.07%) were inside the AD of a single model, and 9143 (4.16%) were inside the AD of only two models.

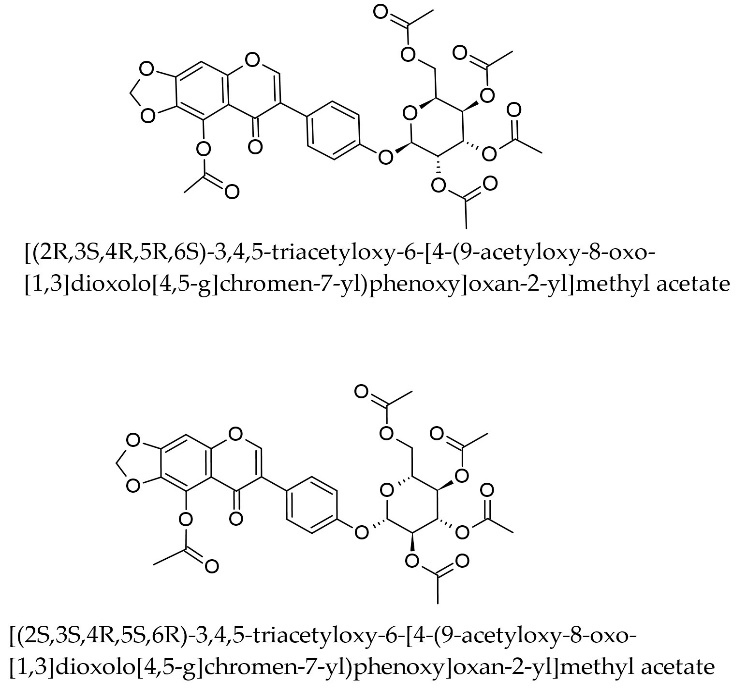
Use case example for herbal extracts

Iqbal Choudhary et al. (2005) reported that an ethanolic extract of *Iris × germanica* L. (rhizomes) reduced all lipid components, including LDL-cholesterol, significantly [30]. The authors neither identified, nor discussed the responsible chemical compounds or the mechanism of action involved, and we could not identify published further research to clarify this aspect. We therefore were interested in assessing whether compounds biosynthesized by *Iris × germanica* have the ability to inhibit HMGCoA-reductase. For this purpose, we downloaded all chemical compounds reported up to date as identified in this species from the Lotus database of natural compounds, getting a data set of 129 compounds which were virtually screened using our selected models in a similar manner with those from ZINC. Seven compounds from this data set were outside of the AD of all six models and 12 compounds were inside the AD of a single model among the six. 60 (46.5%) compounds were inside the AD of all six models and 38 (29.5%) were inside the AD of five out of the six models. No compounds of this data set had a predicted IC50 less than 10 nM (Fig. 7), and only two compounds were predicted by the models to have an IC50 less than 100 nM, both being stereoisomers of the same acetylated isoflavone basic structure: [(2R,3S,4R,5R,6S)-3,4,5-triacetyloxy-6-[4-(9-acetyloxy-8-oxo-[1,3]dioxolo[4,5-g]chromen-7-yl)phenoxy]oxan-2-yl]methyl acetate and [(2S,3S,4R,5S,6R)-3,4,5-triacetyloxy-6-[4-(9-acetyloxy-8-oxo-[1,3]dioxolo[4,5-g]chromen-7-yl)phenoxy]oxan-2-yl]methyl acetate (Fig. 8), with a predicted mean IC50 of 25.07 nM (RSD 11.57%; the median of the predictions for these compounds was about 36.78 nM, and the predicted IC50 made by the svm-based ensemble model was 60.26 nM).



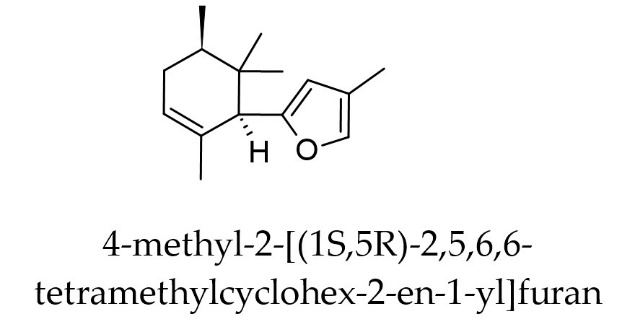
**Figure 7**. Histogram of the predicted pIC50 values for the chemical compounds reported in *Iris × germanica* L.

**Figure 8**. Two acetylated isoflavonoids from the rhizome of *Iris × germanica* predicted to be highly active against HMGCoA-reductase.



The second most active compound of *Iris × germanica* predicted by the six models was 4-methyl-2-[(1S,5R)-2,5,6,6-tetramethylcyclohex-2-en-1-yl]furan (Fig. 9), a sesquiterpene derivative with a median predicted IC50 of 162 nM, and a predicted IC50 by the svm-based

ensemble model of 595 nM. However, the RSD for the four predictions inside AD in this case was relatively large (27.34%).



**Figure 9**. A sesquiterpene derivative from *Iris × germanica* L. with a predicted IC50 of 37.2 nM.

There were also a number of additional compounds for which the median of the predicted IC50 by the individual models or the predicted IC50 by the svm-based ensemble model were under 1 μM and they could also contribute to the observed effect. They are shown in Table 8. Most of them belong to the isoflavonoid group; a few such additional compounds are flavonoids, terpenoids, and xanthonoids.

Table 8. Compounds predicted to have IC50 values under 1 μM (but > 100 nM)

| No. | Compound | IC50\* (μM) | IC50\*\* (μM) |
| --- | --- | --- | --- |
| Isoflavonoids | | |  |
| 1 | irigenin (5,7,3'-trihydroxy-6,4',5'-trimethoxyisoflavone) | 0.56 | 1.37 |
| 2 | tectoridin (shekanin; 4',5-dihydro-6-methoxy-7-(o-glucoside)isoflavone) | 0.84 | 0.72 |
| 3 | irisolidone (4'-O-methyltectorigenin) | 0.53 | 1.24 |
| 4 | iristectorin A | 0.89 | 0.82 |
| 5 | iristectorigenin B | 0.54 | 1.12 |
| 6 | homotectoridin | 0.87 | 0.70 |
| 7 | germanaism A | 0.52 |  |
| 8 | irilone 4'-O-glucoside | 0.53 | 0.73 |
| 9 | germanaism B | 0.64 | 0.80 |
| 10 | germanaism A | 0.52 | 0.95 |
| 11 | Kakkalidone (irisolidone 7-O-beta-D-glucoside and its stereoisomers) | 0.59 | 0.75 |
| 12 | homotectoridin | 0.87 |  |
| 13 | irisflorentin | 1.73 | 0.80 |
| 14 | pratensein 7-O-glucopyranoside | 2.08 | 0.82 |
| 15 | germanaism G | 2.34 | 0.82 |
| 16 | 3-(3-hydroxy-4,5-dimethoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one | 1.24 | 0.69 |
| 17 | 5-hydroxy-3-(3-hydroxy-4,5-dimethoxyphenyl)-7-[(2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one | 1.41 | 0.78 |
| 18 | germanaism D | 2.44 | 0.85 |
| flavonoids | | |  |
| 19 | isoswertiajaponin | 0.83 | 0.97 |
| 20 | swertisin (flavocommelitin, 6-C-glucopyranosyl-7-O-methylapigenin) | 1.24 | 0.84 |
| 21 | isoswertisin (isoflavocommelitin, 7-O-methylvitexin) | 1.07 | 0.85 |
| 22 | embigenin | 1.30 | 0.66 |
| terpenoids | | |  |
| 23 | iriflorentan (2Z-2-[(2R,3S,4S)-4-hydroxy-3-(hydroxymethyl)-2-(3-hydroxypropyl)-4-methyl-3-[(3E,5E)-4-methyl-6-[(1R,3S)-2,2,3-trimethyl-6-methylidenecyclohexyl]hexa-3,5-dienyl]cyclohexylidene]propanal) | 0.62 | 1.51 |
| 24 | germanical C (2-[4-hydroxy-3-(hydroxymethyl)-2-(3-hydroxypropyl)-4-methyl-3-[4-methyl-6-(2,5,6,6-tetramethylcyclohex-2-en-1-yl)hexa-3,5-dien-1-yl]cyclohexylidene]propanal) | 0.76 | 1.65 |
| 25 | irisgermanical B (2-[4-hydroxy-3-(hydroxymethyl)-2-(3-hydroxypropyl)-4-methyl-3-[4-methyl-6-(2,2,3-trimethyl-6-methylidenecyclohexyl)hexa-3,5-dien-1-yl]cyclohexylidene]propanal) | 0.62 | 1.51 |
|  | xanthonoids |  |  |
| 26 | mangiferin | 1.68 | 0.91 |
| 27 | irisxanthone | 1.84 | 0.98 |
| 28 | isomangiferin | 2.49 | 0.94 |

**\*** Median IC50 value for each compound, calculated using predictions only from models where the compound falls within the AD

\*\* IC50 values predicted by the ensemble support vector machine model

3. Discussion

The QSAR models reported here were built with 2D descriptors, and not 3D. Although it might be tempting to think that 2D descriptors are inferior to 3D ones, in the past it has been shown that across a number of data sets consisting of biologically active compounds, 2D descriptors were able to provide better discrimination among compounds than 3D descriptors [35]. This was three decades ago, and with the progress made in compound alignment and molecular encoding, today this might not be true anymore and 3D-QSAR techniques have multiple strengths, the 2D-QSAR approach still has a number of advantages: it is simpler, faster and best suited for analyzing many compounds and screening large molecular databases [36]. Moreover, the performance of 2D-QSAR models can often be very similar to that of 3D-QSAR models [37]. We therefore were focused on developing a set of valid global 2D QSAR models to use for virtual screening purposes, employing two sets of molecular descriptors: the MACCS keys and a variety of molecular descriptors computed by Alvadesc.

Despite their simplicity, the MACCS keys could be used to build a model whose performance was similar to that of models built with more sophisticated descriptors. This is in line with the findings of Brown and Martin that MACCS keys achieve encoding the highest amount of content about a variety of properties relevant for the interaction with biological targets, such as hydrophobicity, static electricity, steric interactions, dispersion interactions, and intermolecular bonding [35]. In classification models, MACCS (as well as PubChem) fingerprints have been shown to perform better than other fingerprints [38] and in our regression models MACCS yielded results only slightly inferior to the best models constructed with various sets of molecular descriptors.

Among the molecular descriptors, only two pooled sets resulted in models with a reasonably good performance, as defined in this paper: a pooled set consisting of 2D autocorrelations, 2D-matrix based descriptors, and Burden eigenvalues (one model) and another consisting of functional group counts, atom-centered fragments, atom-type E-state indices, and pharmacophore descriptors (four models).

Previously, 2D-autocorrelations have been used successfully to build QSAR models for the bioconcentration factor [39], radical scavenging activity [40], muscle relaxant activity [41], matrix metalloproteinase inhibition and others [42,43]. This family of descriptors is relatively easy to compute and is based on summations of different autocorrelation functions (at different lags) and encodes information about the topology of the molecule or of certain parts of the molecule, as well as certain atomic properties corresponding to that topology [42]. 2D-matrix based descriptors comprise a heterogenous collection of descriptors calculated using several matrices: adjacency matrix, topological distance matrix, Laplace matrix, chi matrix, reciprocal squared distance matrix, detour matrix, Barysz matrix, and Burden matrix. More specifically, the best performing models included several descriptors calculated from the chi matrix (SM3\_X), Laplace matrix (TI2\_L ), and Burden matrix (SpMax\_B(p), VE1sign\_B(s)).

MATS3e (Moran autocorrelation of lag 3 weighted by Sanderson electronegativity) has been often identified in the literature as a “potent” descriptor able to characterize a variety of ligand-protein interactions [44]. It has been speculated that compounds with higher values of this descriptor have higher electronegative functionalities that favor forming hydrogen-bond interactions with amino acid residues of the target protein active site [45]. In the case of our models, more negative values of MATS3e tended to associate with a more pronounced inhibitory effect. From a structural point of view, greater negative values tend to indicate greater disparities in electronegativity between atoms that are separated by 3 bonds. Its values tended to correlate well with MATS3s (Moran autocorrelation of lag 3 weighted by I-state).

MATS1p (Moran autocorrelation of lag 1 weighted by polarizability) encodes information about the distribution of polarizability in a molecule, namely between neighboring atoms (lag 1). In the literature it was reported to correlate positively with the inhibitory activity of imidazole derivatives on glutaminyl cyclase[53] or the inhibitory activity on type I fatty acid synthase [54]. Within our model, MATS1p exhibited an inverted U-shaped relationship with the inhibitory activity on HMGCoA reductase.

SpMax\_B(p) (leading eigenvalue from Bur-den matrix weighted by polarizability) is a less intuitive descriptor, being a leading eigenvalue derived from the Burden matrix (a mathematical instrument of representing the interactions between molecule atoms) and weighted by polarizability. It may be thought of as reflecting the contributions of all atoms in the molecule, and thus reflecting the diversity or similarity of a dataset or database [46]. Its correlation with the inhibitory effect on HMGCoA reductase has an inverted-U-shape (concave-down shape).

SpMin1\_Bh(e) (smallest eigenvalue n. 1 of Burden matrix weighted by Sanderson electronegativity) belongs to the Burden eigenvalues and seems to have been little used in published QSAR models up to date. One study reported that it is negatively correlated with the binding affinity for the bacterian *LasR* protein [49]. We found that in relationship with HMGCo-A inhibitors, it exhibit a negative association, with an asymmetric inverted U-shape.

VE1sign\_B(s) (coefficient sum of the last eigenvector from Burden matrix weighted by I-State) is a 2D-matrix based descriptor that has been rarely reported as important in QSAR studies. In a recent study it was found as the second most important descriptor in describing the activity of aromatase inhibitors [47]. In a QSAR study describing toxicity of chemical compounds on the springtail *Folsomia candida*, higher (positive) values of this descriptor tended to associate with more toxicity [48]. In our model built with Set 2 of descriptors, higher values were predictive of lower activity.

SM3\_X (spectral moment of order 3 from chi matrix) was not up to date reported as an important descriptor in the QSAR literature. It provide information on the structural complexity of the molecule and it could reflect certain electronic properties of the molecule. Whereas SM3\_X is less intuitive and does not lend to a simple interpretation, at least in our data set it was highly correlated with SM5\_X, as well as with the number of 3-membered rings and the distance/detour ring index of order 3/ SRW03 (self-returning walk count of order 3), meaning that the presence of 3-membered rings (e.g. epoxides, aziridines, or cyclopropane groups) tend to associate with lower pIC50 (i.e. less active compounds). In a recent study it was found that nR03 (a descriptor highly correlated with SM3\_X) tended to decrease the toxicity of chemical compounds on *Daphnia magna* [50]. In the regression model constructed using the Set 2 descriptors (2D matrix-based descriptors, 2D autocorrelations, and Burden eigenvalues) (model no.12 in Table 1), it showed a negative correlation with pIC50.

GATS5v (Geary autocorrelation of lag 5 weighted by van der Waals volume) is a 2D autocorelation descriptor encoding information about molecular size, shape, steric effects and distribution of the van der Waals volume across the molecule, particularly for atoms separated by 5 bonds (lag 5). It has been shown to be an important predictor for the antagonistic activity of non-peptide compounds against the CXCR2 chemokine receptor [51] as well as for the antiproliferative of 3,4-dihydropyrimidin-2-(1H)-thiones [52]. In our model, higher values of GATS5v were associated with higher inhibitory properties on HMGCoA reductase.

JGI5 (mean topological charge index of order 5) is a topological index, whose values will tend to increase with the complexity of the molecular structure (more branching, more ring systems, increased number of heteroatoms). In the literature, JGI5 has been shown to have a positive associated with the antimalarial activity [55] or a stronger antioxidant activity [56]. In our model, higher values of JGI5 associated with a higher inhibitory activity on HMGCoA reductase.

TI2\_L (second Mohar index from Laplace matrix) is calculate as the inverse of the smallest non-null eigenvalue of the Laplace matrix, weighted by the amount of heavy (non-hydrogen) atoms. It ignores the existence of heteroatoms in a molecule, but it is sensitive to structural properties like branching and ring presence. Its value rises with the amount of non-hydrogen atoms present. In a set of molecules with the same size, it distinguishes between linear chains (higher values) and branched/cyclic structures (lower values). It has been shown to be useful in the prediction of molecule biodegradability [57] and placental barrier permeability [58]. Higher values of TI2\_L are associated with a lower inhibitory activity, indicating that a certain degree of branching or cyclicity is necessary for the HMGCoA reductase inhibition.

Among the atom-centered fragments, C-034 (R–CR..X, where X is a non-carbon heavy atom, whereas R is an aliphatic group) and C-003 (a CHR3 group) were shown to correlate with the inhibitory activity on HMGCoA. C-034 correlated well with several other descriptors (see Table 6), among which the number of number of pyrrole rings, itself selected as a useful descriptor in other models. Higher values of C-034 associated with increased activity. C-034 has been reported in the literature to be useful in predicting the glutaminyl cyclase inhibitory activity for imidazole derivatives [53]. C-033 (R–CH..X), has a similar effect as C-034. In previously published model, it was shown to be the most important in predicting herbicidal activity [59], but also in predicting radiosenzitizing properties [60]. C-003 was found to be relevant for the binding of small molecules to the active site or the pockets of vasoactive metalloproteases [61] and in predicting the inhibitory activity of biphenylsulfonamides on aggrecanase-1 [55]. In our models, a value of 3 or less was associated with lower activity on HMG-CoA reductase, whereas values of 4 or 5 were associated with higher activity.

C-001 (corresponding to the number of methyl groups, which can induce a certain degree of lipophilicity [70]) has been used in published QSAR models for acetylcholinesterase inhibitors [71]. Both C-001 and the number of pyrrole rings (nPyrroles) had a weak positive association with the HMG-CoA reductase inhibitory activity. The number of pyrrole rings seems not to have been previously selected in published QSAR models. C-002, an atom-centered fragment describing the number of CH2R2 fragments had a sawtooth-like relationship with the HMG-CoA reductase inhibitory activity, the strongest activity being observed at the lowest value for these fragments. In published QSAR models, this descriptor was used in modelling linear retention indices for essential oil ingredients [72] and the antagonistic activity of chemical compounds against the growth hormone secretagogue receptor [73]. C-006 (CH2RX, i.e. numbers of a carbon atom joined to two hydrogen atoms, a heteroatom, and another carbon atom) is a descriptor that has been used in previous research to model the MMP-13 inhibitory activity [74], CK2 inhibitory activity [75] or the aqueous solubility of chemical compounds [76]. In our models, a higher value for this descriptor tended to associated with lower inhibitory activity on HMG-CoA-reductase.

H-046 (defined as H attached to C0(sp3) no X attached to next C, i.e. a hydrogen atom joined to a carbon atom that is saturated (sp3 hybridized), with the subsequent carbon atom unattached to a heteroatom), is an atom-centred fragment descriptor that has been used to model binding of ligands to the 5-HT6 receptor [77], the inhibitory activity against CDK2 [78], or the PPARγ agonistic activity [79]. In our models, a sawtooth-like curve represented the link between this descriptor and the inhibitory activity of HMG-CoA-reductase, with highest activity observed at the lowest values. H-053 (defined as H attached to C0(sp3) with 2X attached to next C; in other words, H - C - C(XX), where: C is an sp3 carbon and C(XX) represents the neighboring carbon with two heteroatoms) is another atom-centred fragment that in previous research has been used in the QSAR modeling of the serotonin 1A and adrenalin α1-adrenergic receptor binding activity [80], of human beta-secretase inhibitors [81], and of the antibacterial activity for pleuromutilin derivatives [82]. In our models, a flattened inverted U-shape was observed for this descriptor in relationship with the HMG-CoA reductase inhibitory activity. The O-056 descriptor (number of alcohol fragments) was negatively associated with the HMG-CoA reductase inhibitory activity. It has been used in previously published research to model the odor aroma of wine components [83] or the antimicrobial activity of newly synthesized chemical compounds [84]. The number of pyrimidines (nPyrimidines) positively correlated with the HMG-CoA reductase inhibition. In the past, it has been shown that this descriptor also correlates with hepatotoxicity [85] and with the CYP2C9-drug interaction [86].

nCrt (number of ring tertiary C) belong to the functional group counts and was previously reported to be a useful predictor of P-glycoprotein substrates [62]. A value of zero for nCrt was associated with higher HMG-CoA reductase inhibitory activity, whereas values of 1 or higher, were associated with lower activity. NsF (number of atoms of type sF, i.e. single bond fluoride) was also relevant for the HMG-CoA reductase inhibition, fluorinated molecules having a higher activity. This is an aspect that has already been discussed in the literature, a fluorine substituent in the pyrrole core of atorvastatin being more effective than other ligands, and fluorine substituents in the hydrophilic side-chain of other statins having stronger inhibitory effects on the target enzyme [63].

nCconj (the number of non-aromatic conjugated carbon atoms, C(sp2)), is a descriptor indicating the count of carbon atoms in a molecule that are sp2 hybridized (having a planar structure with one double bond), involved in a conjugated system, and not part of an aromatic ring. It has been shown to be useful in predicting the larvicidal activity of terpenoids against *Culex quinquefasciatus* [64] or the activity against *Trypanosoma cruzi*, the causative agent of the Chagas disease [65]. In our models, a higher number of non-aromatic conjugated carbon atoms was linked to greater inhibitory activity on HMG-CoA reductase.

SaaaC is an E-state descriptor, more precisely the sum of aaaC E-states, i.e. aromatic carbon atoms that have no hydrogen atoms attached and are connected to three other aromatic atoms; the higher its value, the higher the reactivity and number of those carbon atoms. SaaaC has been shown to be negatively associated with the inhibitory activity against bacterial biofilms [66]. The same kind of relationship has also been observed in our models (lower values of this descriptor are associated with an increase level of activity). Conversely, greater values of SaaCH (the sum of aaCH E-states, i.e. all the non-substituted carbon atoms in an aromatic molecule) were correlated with slightly elevated activity. This descriptor was previously used in modelling algal toxicity [67] and cytotoxicity on the MCF-7 breast cancer cell line [68]. SssCH2 (sum of ssCH2 E-states, i.e. electrotopological states of a methylene group attached to the remainder of the molecule through single bonds) has been useful in modelling the histone deacetylase inhibition activity [69] and in modelling the critical micelle concentration (CMC) for anionic surfactants [68]. A somewhat reduced level of activity was linked to higher values of this descriptor in our models.

CATS2D\_04\_AA (CATS2D Acceptor-Acceptor at lag 04) belongs to the sub-block of CATS (Chemically Advanced Template Search) 2D descriptors, in the pharmacophore descriptor block. A value of 3 or higher is associated with a stronger inhibitory activity on HMG-CoA reductase. In a recent paper it was shown that CATS2D\_04\_AA is an important predictor of blood–brain barrier permeability [87], as well of skin permeability [88] for different substances. CATS2D\_04\_DA (CATS2D Donor-Acceptor at lag 04) belongs to the same descriptor block and (at least in our data set) was well correlated with CATS2D\_04\_AA. It has been employed in previous studies to construct quantitative structure toxicity–toxicity relationship models [89] and in modelling the inhibitory activity of chemical compounds against the MAO-B enzyme [90]. CATS2D\_07\_DA (CATS2D Donor-Acceptor at lag 07, i.e. at a seven bonds distance) has been used in modelling O6-methylguanine-DNA methyltransferase inhibitory activity, where higher values correlated with lower activity [91]; the same type of relationship was also seen in our models. CATS2D\_07\_DL (CATS2D Donor-Lipophilic at lag 07) has been used in published QSAR models for *Aedes aegypti* repellents [92], models for antioxidant activity of coumarin derivatives [93], or the anticancer activity of N-(aryl/heteroaryl)-4-(1H-pyrrol-1-yl)-benzenesulfonamide derivatives [94]. In our models, the inhibitory activity against the HMG-CoA reductase was associated with higher values of this descriptor. CATS2D\_06\_AL (CATS2D Acceptor-Lipophilic at lag 06) is a descriptor that has been little used up to date in QSAR models; we have identified only a model where it was used in the chemometric analysis of drug groups with various pharmacological activities [95] and a model where it was used in modeling the antioxidant effects (TEAC) of chemical compounds [96]. Larger values of this descriptor tended to associated with lower inhibitory activity on HMG-CoA reductase. CATS2D\_03\_DL (CATS2D Donor-Lipophilic at lag 03) has been used to model toxicity of chemical compounds against bees [97] and the binding aﬃnity of substances with endocrine disruptor properties [98], whereas CATS2D\_09\_DL (CATS2D Donor-Lipophilic at lag 09) seems to have not been part of QSAR models published up to date. An increase in activity was associated in our models with lower values of these two descriptors. CATS2D\_02\_AL (CATS2D acceptor-lipophilic at lag 02, i.e. two bonds apart) is another pharmacophore descriptor, who has been used in modelling the biological activities of SGLT2 inhibitors [99] and the multiple endpoint acute toxicity of chemical compounds (higher values, higher toxicities) [100].

Shannon entropy descriptors were first proposed in 2006 [101], and have not been extensively used up to date in QSAR models. SHED\_AN (Shannon entropy descriptor, acceptor-negative) is a descriptor that offers information regarding the spatial arrangement of acceptor and negative atoms inside the molecule. Up to date it has been used in models predicting the blood-brain barrier permeability [102]. Higher SHED\_AN values in our models were linked to marginally lower activity. Similarly, SHED\_AA (Shannon entropy descriptor, acceptor-acceptor) is an expression of the diversity or uniformity of the acceptor-acceptor interactions (acceptors being generally electronegative atoms, e.g. halogens, oxygen, nitrogen). Lower values of SHED\_AA was associated with higher HMG-Co-A inhibitory activity in our research.

As shown in the results, the virtual screening of almost 220,000 chemical compounds (mostly natural) from the ZINC 15 database predicted for only 237 compounds a mean of pIC50 reliable predictions (i.e. inside the AD) equal to or higher than 8, and 287 compounds a the median of reliable predictions higher than 8 (i.e. had IC50 values equal to or lower than 10 nM). Using the svm-based ensemble model, a number of 168 compounds (about 0.08%) had predicted IC50 values lower than 10 nM. In a recent paper, Athista et al. (2023) reported on virtual screening to identify HMG-Co-A reductase inhibitors using ligand-protein docking and their predicted active compounds rate was of 22 natural compounds out of 558 compounds tested, i.e. 3.94% [103].

We have also shown how such QSAR models can be used to enhance understanding of non-clinical experiments performed with herbal extracts where a pharmacological mechanism of the anti-hypercholesterolemiant effect has not been explored. In our use case example, we have identified a number of natural products from *Iris germanica* L. that could explain the ability of an extract obtained from the rhizomes of this species to reduce LDL-cholesterol. Among the compounds predicted by our models to be active was mangierin. For this substance, the median of the IC50 values predicted by the four best-performing models for which the substance was within the AD was 1.68 μM, whereas experimentally an inhibition constant of 3 ± 0.2 μM was determined [104], which seems in quite good agreement. The ensemble model based on svm estimated an IC50 of 0.90 μM, which is also close to the experimental inhibition value. For irisolidone IC50 values of 0.53 or 1.24 μM were predicted by our models, whereas in one experiment, an IC50 of 36 μM was estimated [105]. Such examples for which we have found experimental evidence verifying the predicted activity tend to confirm the validity of the models and their usefulness in this setting.

4. Materials and Methods

4.1. Data set

A set of 1170 inhbitors of human HMG-CoA reductase, whose activity was assessed based on their half-maximal inhibitory concentrations values (IC50), were downloaded from ChEMBL (target ID CHEMBL402) [106]. The SMILE chemical formulas were carefully examined manually and inorganic or excessively simple compounds (e.g. sodium arsenite, strontium chloride hexahydrate, thioacetamide etc) were removed from the data set, as were polymers (e.g. macrogol), mixtures or other compounds without a defined chemical structure. ChemAxon Standardizer 18.8.0 (ChemAxon, Budapest, Hungary) was used to standardize the chemical structure of the data set compounds, applying the following operations: stripping salts, neutralization, tautomerization, aromatization, clean 2D and adding explicit hydrogens (in this order). Following standardization, duplicate compounds were removed from the data set and their IC50 values were replaced by the median (as this is more relevant than mean in the presence of outliers). Compounds available as both acid and salt forms (e.g., lovastatin and lovastatin sodium, maduramicin and maduramicin ammonium) were treated as duplicates, retaining the acid form. DataWarrior (v. 6.1.0) [107], FlareTM for Academics, v.7.0 (Cresset®, Litlington, Cambridgeshire, UK), and the computing and programming environment R, v. 4.3.1[108] were used for this purpose. Following the pre-processing operations, the final data set consisted of 1042 compounds (available with their chemical structures in SMILES notation in Table S1); their IC50 values varied between 0.002 nM and 1,500,000 nM, while molecular weight varied between 32 g mol−1 to 2297 g mol−1. Among the 1042 compounds, numeric IC50 values were available only for 227 compounds, whereas for the large majority of the data set IC50 values were not accessible, and thus not apt for use in building regression models. For modeling purposes, the IC50 values were converted to pIC50 by taking the negative logarithm (log10) of the corresponding molar concentration. The 227 compounds were randomly split in training and testing data sets in a 3:1 ratio (170 and 57 compounds, respectively).

Molecular fingerprint calculation

R package “Rcpi” (an open source library) [109] was used to calculate MACCS keys (166 bits), under Rstudio, v. 2021.09.1, Build 372 [110]. Molecular fingerprints are means of molecular structure representation which encode the presence (attributing a value of 1) or absence (attributing a value of 0) of certain fragments/substructures in a chemical molecule [111]. MACCS fingerprints were initially intended to be used for substructure searching [112], but have later become widely used in QSAR modeling and are still relevant for this purpose [113]. AlvaDesc software [114] was used to compute used to calculate 3874 2D molecular descriptors, grouped into 18 blocks (constitutional indices, ring descriptors, topological indices etc).

Chemical space distribution and diversity

To explore the diversity and distribution of the data set compounds in the chemical space we have used two features widely used in the field for this purpose, molecular weight and atomic logP (AlogP, AK Ghose – G.M. Crippen logP) [115], computed by the R package “Rcpi” [109]. We have also examined the fulfillment of the Lipinski’s “rule of five”, as a criterion of “druggability” or “drug-likeness” for the compounds included in the modeling exercise [116], also using the “Rcpi” R package [109]. To assess the data set diversity, the average Tanimoto similarity index (computed in R with the “proxy” R package [117]) was used.

Feature selection, model building and validation

MACCS fingerprints consist of 166 binary features/keys, whereas Alvadesc calculates over 4000 of 1D or 2D descriptors. Both are high numbers that have to be reduced in order to build meaningful models, because of the so-called “curse of dimensionality” which if not appropriately approached, increases the probability of modelling noise and getting useless models [118]. It is accepted that most often only a minor subgroup of all descriptors are likely to carry the information essential to develop good mathematical models with a particular data set [22]. Therefore, feature selection is an important step of the QSAR model building process, and an impressive number of methods and algorithms have been advanced for this purpose. They are classified as either filter methods (faster and less computationally intensive) and wrapper methods (more robust but more time-demanding and computationally intensive) [119].

For the regression models, we have explored the use of six filter methods, through the unified interface “mlr3”[120]: “carscore” (from R package “care” [121]), “correlation”, “cmim” (R package “praznik” [122]), “find\_correlation”, “relief” (R package “FSelectorRcpp” [123]), and “information gain” (R package “FSelectorRcpp” [123]). Feature selection was preceded by removal of constant, quasi-constant 37 features removed) and highly correlated features, for the latter using a correlation cut-off of 0.90 and the “FeatureTerminatoR” [124] R package (36 additional features removed). Feature selection was coupled with hyperparameter search and a 10-fold (and in a small number of cases, 5-fold) cross-validation; this k-fold cross-validation was merely used to improve the filtering methods results and not for the validation of the modeling exercise (external validation and nested cross-validation are described and reported below). We compared the feature filtering methods in groups of three (“carscore”, “correlation”, and “cmim” a fist group, “find\_correlation”, “relief”, and “information gain”, a second one), and the features of the best performing were used to build the regression models. For this purpose the following regression algorithms were used:

* multiple linear regression
* elastic net regression (“glmnet” R package, varying the *alpha* parameter between 0.0001 and 1)
* multivariate adaptive regression splines (“earth” R package)
* k nearest neighbors with various kernels (“kknn” and “fnn” R package)
* Quinlan M5 rule trees (“cubist” R package and “RWeka” R package).
* random forests (“ranger” R package), conditional inference trees and conditional random forests (“partykit”, “sandwich” and “coin” R packages)
* support vector machines (“e1071” R package) and regularized support vector regression (“LiblineaR” R package)
* extreme gradient boosting (“xgboost” R package) and generalized boosting models (“gbm” R package)
* Bayesian Additive Regression Trees (BART) (“BART” R package).

For tree-based algorithms (Quinlan M5 rule trees, random forests, extreme gradient boosting, BART) numerical features were used as such (unscaled) in building and assessing the performance of the models. For the remainder of the algorithms used, features were centered and scaled (using the base R function *scale* within the mlr3 pip pipeline).

To estimate the performance of the model building exercise, we applied a nested-cross validation procedure, using an inner loop of 10 folds and an outer loop of 10 folds and tuning the hyperparameters for each model inside the inner loop. We have used the root mean squared error (RMSE) as a scoring function for tuning and the nested cross-validation R2 (true q2 [125]) as a more easily interpretable performance measure, as well as the concordance correlation coefficient (CCC, computed with the “agRee” R package [126]). We have also applied the models built on the external validation data set and used the R2 and the CCC between the true values and those predicted by the models. The CCC was initially proposed by Lawrence I-Kuei Lin in 1989 as a measure of reproducibility [127] , but was more recently recommended in the field of QSAR as a more conservative metric having the property of being “a true external validation measure” (using no information from the training data set) [128,129]. We rejected models for which the R2 values for the test set were lower than 0.70; therefore, for those models we did not perform a nested-cross validation. To control for the possibility of good performance due to chance associated with a certain seed number, we have repeated the nested cross-validation five times for each model, with different random seeds.

To estimate the risk of chance-correlation, a y-scrambling test (described in the literature as “probably the most powerful validation procedure”) [130] was performed on three of the selected models: the model with the highest R2 value in the nested-cross validation (R2 = 0.75), one among the models with the lowest acceptable R2 values (R2 = 0.70), and one with an intermediate level for R2 value (0.72) (all three models were built with the set 4 of Alvadesc descriptors). For each model the response variable was permuted 20 times, and the whole model building process was repeated from step zero (scaling, feature selection with the relevant methods, nested cross-validation).

To assess feature importance, identify the most important variables associated with the HMGCoA reductase inhibition in the best models, and interpret those models, the “DALEX”[131] and “iml”[132] R packages were used.

Trustworthy QSAR model applications rely on the *applicability domain* (AD), which is defined in large part by the characterization of the interpolation space [133]. For models built using MACCS fingerprints, which are binary variables, the apd\_similarity() function from the “applicable” R package [134] was used to estimate the AD; compounds with a similarity larger than 20% versus the training set were considered inside AD. For the molecular descriptors (computed with the Alvadesc software), the Isolation Forest algorithm was used, as implemented in the “isotree” R package [135], with a number of features randomly selected for splitting (“ntry”) of 10. (The same algorithm is borrowed from the “isotree” by the “applicable” R package).

In order to conduct high-throughput virtual screening for possible inhibitors of HMGCoA reductase, a library of about 220,000 chemical compounds was obtained from the ZINC database. They were downloaded in the SMILES format and the same molecular descriptors were computed using Alvadesc as for the training compounds. We then used the six best performing models to predict the pIC50 values for the screening chemical compounds. We evaluated whether each compound fell or not inside the AD of each model and computed the median and mean of the predictions that could be trusted based on the AD assessment, as well as the relative standard deviation. The latter allows us to understand how widely the predictions have varied among the models whose results were selected for pooling (the molecules being inside of the AD of those models). In order to illustrate a practical application of the models we have also downloaded the chemical structures of all chemical compounds reported as identified in the *Iris germanica* L. species in the Lotus database [136], computed the molecular descriptors and then virtually screened each compound in a similar way with the ZINC compound dataset.

5. Conclusions

We have developed a set of QSAR models for human HMG-CoA reductase inhibitors, employing nested cross-validation as the primary validation method, and utilizing the top-performing models for the virtual screening of approximately 220,000 chemical compounds from the ZINC 15 database. Active substances (IC50 < 100 nM) exhibited molecular weights from 369.4 to 778.1 g mol−1 and ALogP values ranging from 1.4 to 8.4. In contrast, the ten statins displayed molecular weights between 390.5 and 558.6 g mol−1 and ALogP values from 2.1 to 5.5. A number of 300 models were built using various machine learning regression algorithms, feature selection methods, and fingerprints or descriptor datasets. 21 models were selected for their good performance (R2 ≥ 0.70 or CCC ≥ 0.85), among which six met both performance criteria and were used to construct five ensemble models. Employing y-randomization, while feature selection with basic cross-validation yielded satisfactory performance for some models, nested cross-validation revealed significant underperformance across all performance measures, thus confirming the validity of the selected models. Using the DALEX and iml R packages, the descriptors that were most important in explaining HMGCoA inhibition in the six best-performing models were identified. Only 237 of about 220,000 compounds had a mean pIC50 reliable prediction (i.e., within the AD) of 8 or higher, while 287 of the compounds had a median of 8 or higher for reliable predictions (i.e., IC50 values equal to or lower than 10 nM). A total of 168 substances (or roughly 0.08%) had predicted IC50 values less than 10 nM using the svm-based ensemble model. The developed QSAR models can be successfully applied to understand the compounds involved in cholesterol lowering activities of herbal extracts, for instance an extract of *I. germanica* rhizome.

**Supplementary Materials:** The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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