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Drug-likeness Analysis of Traditional Chinese Medicines: Prediction of Drug-likeness Using Machine Learning Approaches

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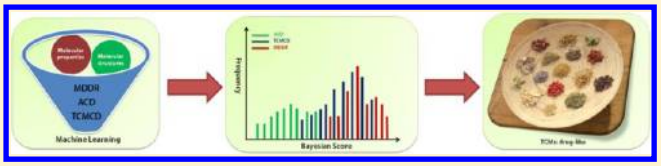
Supporting Information

ABSTRACT: Quantitative or qualitative characterization of the drug-like features of known drugs may help medicinal and computational chemists to select higher quality drug leads from a huge pool of compounds and to improve the efficiency of drug design pipelines. For this purpose, the theoretical models for drug-likeness to discriminate between drug-like and non-drug-like based on molecular physicochemical properties and structural fingerprints were developed by using the naive Bayesian classification (NBC) and recursive partitioning (RP) techniques, and then the drug-likeness of the compounds from the Traditional Chinese Medicine Compound Database (TCMCD) was evaluated. First, the impact of molecular physicochemical properties and structural fingerprints on the prediction accuracy of drug-likeness was examined. We found that, compared with simple molecular properties, structural fingerprints were more essential for the accurate prediction of drug-likeness. Then, a variety of Bayesian classifiers were constructed by changing the ratio of drug-like to non-drug-like molecules and the size of the training set. The results indicate that the prediction accuracy of the Bayesian classifiers was closely related to the size and the degree of the balance of the training set. When a balanced training set was used, the best Bayesian classifier based on 21 physicochemical properties and the LCFP_6 fingerprint set yielded an overall leave-one-out (LOO) cross-validated accuracy of 91.4% for the 140,000 molecules in the training set and 90.9% for the 40,000 molecules in the test set. In addition, the RP classifiers with different maximum depth were constructed and compared with the Bayesian classifiers, and we found that the best Bayesian classifier outperformed the best RP model with respect to overall prediction accuracy. Moreover, the Bayesian classifier employing structural fingerprints highlights the important substructures favorable or unfavorable for drug-likeness, offering extra valuable information for getting high quality lead compounds in the early stage of the drug design/discovery process. Finally, the best Bayesian classifier was used to predict the drug-likeness of 33,961 compounds in TCMCD. Our calculations show that 59.37% of the molecules in TCMCD were identified as drug-like molecules, indicating that traditional Chinese medicines (TCMs) are therefore an excellent source of drug-like molecules. Furthermore, the important structural fingerprints in TCMCD were detected and analyzed. Considering that the pharmacology of TCMCD and MDDR (MDL Drug Data Report) was linked by the important common structural features, the potential pharmacology of the compounds in TCMCD may therefore be annotated by these important structural signatures identified from Bayesian analysis, which may be valuable to promote the development of TCMs.



KEYWORDS: *drug-likeness, traditional Chinese medicine (TCM), naive Bayesian classification, recursive partitioning, machine learning*

INTRODUCTION

It is estimated that the number of molecules in chemical space may be 10^{40}–10^{100}, but the number of molecules registered already is about 2.7×10^7. Moreover, only a small portion of the total chemical molecules are drugs or drug-like molecules.[1] It has been expected that the advent of new techniques, such as high-throughput screening (HTS), combinatorial synthesis, and computer-aided drug design (CADD), can greatly boost the efficiency of drug discovery. However, to our disappointment, from 1980 to 2008, the number of new chemical entities (NMEs) approved by the Food and Drug Administration (FDA) did not increase obviously and even decreased slightly in recent years, suggesting that it still has many difficulties not only to select appropriate lead compounds from the entire chemical space but also to turn the promising leads to marketed drugs. The main reason is that most designed lead compounds cannot achieve a good balance of potency, selectivity, and

Received: April 10, 2012
Revised: May 18, 2012
Accepted: June 27, 2012
Published: June 27, 2012

ADMET (absorption, distribution, metabolism, elimination, and toxicity) profiles. How to change the "time-consuming, expensive, difficult, and inefficient process" of drug discovery is a tough task we are facing now.[2]

Medicinal and computational chemists proposed the concept of "drug-likeness", which is valuable to select more promising lead candidates by evaluating or predicting their drug-likeness in the early stage of drug discovery. Therefore, it is important to understand and develop rules or theoretical models to assess the drug-likeness of chemical molecules.[3−5] At first, the rules of drug-likeness were proposed by analyzing the physicochemical properties of known drug and non-drug databases.[6−8] The most famous drug-likeness filter is Lipinski's "rule-of-five",[7] which suggests that drugs through oral administration should have some similar physicochemical ranges. Contrary to simple molecular physicochemical properties, several structure-based filters have been developed to distinguish drug-like from non-drug-like molecules.[9−14] In sum, these drug-likeness filters based on physicochemical properties and/or structural features are too simple. Moreover, these rules were inferred from the limited known drugs or drug-like molecules and may not cover the chemical space outside the data for training. It has been found that different classes of drugs focus on different chemical space and then the drug-likeness criterion may be different correspondingly.[15] Therefore, these drug-likeness filters or rules may be useful in the early stage of drug discovery to some extent while they should be used cautiously.

To construct more reliable and quantitative theoretical models for drug-likeness, based on larger number of molecular descriptors, machine learning techniques have been used, such as support vector machine (SVM),[16−20] artificial neural networks (ANN),[21,22] recursive partitioning (RP)[23−25] etc. Encouragingly, most prediction models for drug-likeness show good classification accuracy for drugs and non-drugs. Overall, the reliability of some reported prediction models of drug-likeness is questionable because the training set for model building was usually not prepared carefully and the effect of the intrinsic difference of some physicochemical properties between drug-like and non-drug-like molecules was not eliminated.[16,18] In order to guarantee the reliability of the prediction models, the training set for model building should be prepared carefully. In the mean time, the accuracy of the prediction models is closely related to the descriptors and statistical techniques, which should be carefully combined for the best prediction. Moreover, we need to emphasize that the drug databases, such as CMC (Current Medicinal Chemistry), MDDR (MDL Drug Data Report), WDI (World Drug Index) etc., and the nondrug databases, such as ACD (Available Chemicals Directory), are updated frequently, and the latest versions have more entries and may cover wider chemical space.

In this paper, we set out to evaluate the drug-likeness of molecules in the Traditional Chinese Medicine Compound Database (TCMCD). In particular, we tried to develop more reliable classification models of drug-likeness and then evaluate the drug-likeness for TCMCD in a more quantitative way.

MDDR and ACD were used as drug-like and non-drug-like databases, respectively. The impact of molecular descriptors (molecular properties and structural fingerprints), the size and the composition of the training set on the prediction accuracy of the naive Bayesian drug-likeness models were systematically investigated. The best Bayesian classifier gave satisfactory predictions for the molecules in the test set. Moreover, the naive Bayesian classifiers highlight the essential structural features important for drug-likeness, and those structural features were possibly connected to specific pharmacological activities, which are promising for the design or identification of high quality drug leads. Application of the Bayesian classifier to TCMCD suggests that most molecules in TCMCD are drug-like, indicating that TCMCD is a potential source for drug design and discovery. Given the fact that the important fingerprints are related to specific pharmacological activities, the potential pharmacology of the compounds in TCMCD may be inferred by the important fingerprints identified by our models. We expect that our study can provide valuable information to accelerate the development of traditional Chinese medicines (TCMs).

MATERIALS AND METHODS

1. Preparation of the Data Sets of Drug-like and Non-Drug-like Molecules. In order to generate a model that can successfully predict whether a molecule is drug-like or not, molecular databases known to be drug-like and non-drug-like are needed. Here, the MDDR and ACD databases were chosen as representatives for drug-like and non-drug-like databases, respectively. The 3-D structure database of molecules found in TCMs was developed by our group.[26,27] The new version of TCMCD has 63,759 organic molecules identified in more than 5000 herbs in TCMs. We got 142,747 molecules from MDDR, 2,175,382 molecules from ACD, and 63,759 molecules from TCMCD for the following analysis. The protocol to preprocess the three datasets is described in the Supporting Information.

It is well-known that large molecules usually do not have good absorption property, and therefore we set the cutoff for molecular weight to be 600, and the subdatabases were constructed by only choosing molecules with molecular weight less than 600. Comparison study showed that the average molecular weight of the molecules in the ACD subdatabase was about 120 less than that of the molecules in the MDDR subdatabase. It is believed that many molecular properties are dependent on molecular weight, and so in order to construct the prediction models of drug-likeness unrelated to molecular weight, we constructed two subsets of ACD and TCMCD, which have similar molecular weight distribution of the MDDR subdatabase with 123,927 entries. After applying all these preprocessing steps, we extracted a subset of 123,927 molecules from ACD and a subset of 33,961 molecules from TCMCD, and both subsets have similar molecular weight (MW $\leq$ 600) distributions to that of the MDDR subdatabase.

2. Preparation of the Training and Test Sets for Model Development. As mentioned above, both the MDDR and ACD subsets have 123,927 molecules. 40,000 molecules (20,000 from MDDR and 20,000 from ACD) were randomly extracted and served as the test set, and the other molecules (207,854) in the MDDR and ACD subsets served as the source of the training set. How to choose the training set to build a reliable drug-likeness model is an important problem in drug-likeness study. Previous studies have discussed this topic.[16,19,22,23] However, the protocol of choosing the training sets is still confusing. In 1998, Sadowski and Kubinyi selected a training set with 5000 molecules from WDI and 5000 molecules from ACD for the drug-likeness analysis.[22] In 2003, Byvatov et al. used a training set with 4998 drug-like molecules from WDI and 4210 non-drug-like molecules from ACD.[16] Byvatov et al. claimed that the number of molecules in the ACD was much larger than that in the WDI, so the ratio of

the drug-like molecules from the WDI to the non-drug-like molecules from the ACD was skewed from the original 1:4.4 to almost 1:1. In our work, in order to understand how the size of the training set and the ratio of drug-like to non-drug-like molecules influence the classification accuracy of the drug-likeness models, multiple classification models were developed based on different training sets.

First, balanced training sets were prepared: subsets of 10,000 molecules were randomly extracted from both ACD and MDDR, giving a balanced training set of 20,000 molecules in total (first training set); then, more training sets were generated by adding 20,000 (10,000 from MDDR and 10,000 from ACD) randomly selected molecules from the MDDR and ACD subsets to the largest existing training set each time. The last training set contains all the entries in the ACD and MDDR subsets excluding those entering the test set. In total, 10 training sets were prepared. The Bayesian classifiers based on these training sets were built and validated by the same test set. Based on the calculations, the impact of the size of the training set on prediction accuracy was evaluated.

In the following, the unbalanced training and test sets were generated. From the above balanced test set with 40,000 molecules, 2000 molecules randomly selected from MDDR and 4000 to 18000 molecules randomly selected from ACD with a step of 2000 were merged into the unbalanced test sets, and the ratio of drug-like to non-drug-like molecules changed from 1:2 to 1:9. From the molecules outside of the balanced test set, 10,000 drug-like molecules randomly selected from MDDR and 20000 to 90000 molecules from ACD were used to build the unbalanced training sets. For each training and testing cycle, the ratio of the drug-like to non-drug-like molecules in the training set was the same as that in the test set. Based on the calculation results, the impact of the unbalanced degree of the training set on prediction accuracy was evaluated.

3. Calculation of Molecular Descriptors. Here, 21 molecular physicochemical properties widely used in drug-likeness and ADME prediction were chosen.[28,29] The descriptions of the 21 molecular descriptors are listed in Table S1 in the Supporting Information. These descriptors include octanol/water partition coefficient (Alog*P*), the apparent partition coefficient at pH = 7.4 (log*D*), molecular solubility (log*S*), molecular weight (MW), molecular fractional polar surface area (MFPSA), molecular polar surface area (MPSA), solvent accessible surface area (SASA), the number of hydrogen bond acceptors (n_{HBA}), the number of hydrogen bond donors (n_{HBD}), the sum of oxygen and nitrogen atoms (n_{O+N}), the number of aromatic bonds(n_{AB}), the number of aromatic rings (n_{AR}), the number of rings (n_R), the number of three rings (n_{R3}), the number of four rings (n_{R4}), the number of five rings (n_{R5}), the number of six rings (n_{R6}), the number of seven rings (n_{R7}), the number of eight rings (n_{R8}), the number of nine rings and more (n_{R9+}), and the number of rotatable bonds (n_{Rot}). All the descriptors were calculated using Discovery Studio molecular simulation package (version 2.5).[30]

4. Calculation of Molecular Fingerprints. The SciTegic extended-connectivity fingerprints (ECFP, FCFP, and LCFP) and Daylight-style path-based fingerprints (EPFP, FPFP, and LPFP) were used to characterize the substructural features of the studied molecules.[31,32] It should be noted that a fingerprint class is followed by an underscore and the maximum distance. For example, a functional class extended-connectivity fingerprint of maximum diameter 6 generates a property named FCFP_6. Here for each fingerprint class, two diameters, 4 and 6, were considered in our analysis. The smaller diameter, 2, was not used because the structural fragments based on the diameter of 2 are so small and general. The fingerprints represent a much larger set of features than a set of predefined substructures. Furthermore, these fingerprints do not need to be preselected or predefined because they are generated directly from the molecules. Therefore, novel molecular classes are as easily handled as the common classes. The structural fingerprints were generated by using Discovery Studio molecular simulation package.[30]

5. Naive Bayesian Classifiers of Drug-likeness. The naive Bayesian classification technique was used to develop classifiers to discriminate between drug-like and non-drug-like. Bayesian classification can process large amounts of data, can learn fast, and is tolerant of random noise. Moreover, naive Bayesian classification only requires a small amount of training data to estimate the parameters (means and variances of the variables) necessary for classification. Each compound was categorized into drug-like (+) or non-drug-like (−) class, and a vector $\mathbf{f} = \langle f_1, f_2, ..., f_n \rangle$ was prepared, where $f_1, f_2, ..., f_n$ are the calculated values for the n feature variables $F_1, F_2, ..., F_n$ (molecular properties and fingerprints). Then using Bayes's theorem, we get

$$p(C|F_1, F_2, ..., F_n) = \frac{p(C)p(F_1, ..., F_n|C)}{p(F_1, ..., F_n)} \quad (1)$$

where C refers to a compound's class, $p(C|F_1,F_2, ...,F_n)$ is the posterior probability of the compound class, $p(C)$, the prior probability, is induced from the training set, $p(F_1,...,F_n|C)$ is the probability that a compound has certain descriptors given that it is drug-like or non-drug-like, and $p(F_1,...,F_n)$ is the marginal probability that given the descriptors will occur in the data set. The three probabilities on the right side of eq 1 can be learned from a training set containing a number of drug-like and non-drug-like molecules. The mathematical procedure to train a naive Bayesian classifier was described previously.[31,33] The naive Bayesian classifiers were developed in Discovery Studio molecular simulation package.[30]

6. Recursive Partitioning Models of Drug-likeness. The classifiers represented by decision trees were developed by RP and compared with the Bayesian classifiers. The RP technique mimics the human learning and classification process by splitting a data set into smaller and smaller subsets by using a set of hierarchical rules, and at each level the data have higher purity with regard to a property of interest than the one above.[31,34] The result of a RP drug-likeness model is represented by a "decision tree" or "graph" that divides the studied samples into smaller and smaller groups (every group is called a node). The partition procedure is continued until a particular selected predictor is smaller than a predefined cutoff. In our study, 5-fold cross-validation was used to determine the degree of pruning required for the best predictive performance. Considering the huge number of molecules in training, the minimum samples per node was set to 30, maximum knots per property was set to 20, and the maximum tree depth was changed from 5 to 15 with a step of 1 in order to evaluate the effect of tree depth on predictions. We compared the best RP model with the Bayesian classifier based on the same molecular descriptors, training sets, and test sets.

7. Validation of the Prediction Accuracies of the RP and Bayesian Models. For all of the naive Bayesian and RP drug-likeness classifiers, the true positives (TP), true negatives

Table 1. The Performance of the Bayesian Classifiers Trained with the Smallest Balanced Training Set

	training set					test set				
descriptors	SE	SP	Q_+	Q_-	C	SE	SP	Q_+	Q_-	C
MP[a]	0.690	0.586	0.625	0.654	0.277	0.692	0.577	0.621	0.652	0.271
MP+ECFP_4	0.874	0.833	0.840	0.869	0.708	0.864	0.822	0.829	0.858	0.687
MP+EPFP_4	0.801	0.678	0.713	0.773	0.482	0.802	0.673	0.710	0.772	0.479
MP+FCFP_4	0.824	0.789	0.795	0.818	0.613	0.815	0.785	0.791	0.810	0.601
MP+FPFP_4	0.754	0.710	0.722	0.742	0.464	0.752	0.714	0.724	0.742	0.466
MP+LCFP_4	0.878	0.838	0.844	0.873	0.716	0.865	0.832	0.837	0.860	0.697
MP+LPFP_4	0.835	0.834	0.834	0.834	0.669	0.823	0.824	0.824	0.823	0.647
MP+ECFP_6	0.873	0.876	0.875	0.873	0.748	0.858	0.864	0.864	0.859	0.722
MP+EPFP_6	0.782	0.759	0.764	0.777	0.541	0.779	0.759	0.764	0.774	0.538
MP+FCFP_6	0.853	0.844	0.846	0.852	0.698	0.843	0.837	0.838	0.842	0.680
MP+FPFP_6	0.751	0.764	0.761	0.754	0.514	0.747	0.764	0.760	0.751	0.511
MP+LCFP_6	0.893	0.860	0.864	0.889	0.753	0.877	0.850	0.854	0.874	0.728
MP+FCFP_4	0.824	0.788	0.795	0.818	0.613	0.815	0.785	0.791	0.810	0.601

[a]MP represents molecular physicochemical properties.

(TN), false positives (FP), and false negatives (FN) were calculated. The predictive performance of a drug-likeness model was assessed by sensitivity, SE = TP/(TP + FN); specificity, SP = TN/(TN + FP); prediction accuracy of drug-like molecules, Q_+ = TP/(TP + FP); prediction accuracy of non-drug-like molecules, Q_- = TN/(TN + FN); Matthews correlation coefficient, $C = (TP \times TN - FN \times FP)/[(TP + FN)(TP + FP)(TN + FN)(TN + FP)]^{1/2}$. The Matthews correlation coefficient C ranges from 0 to 1, and a perfect classification gives a correlation coefficient value of 1.

RESULTS AND DISCUSSION

1. Naive Bayesian Classifiers Based on Balanced Training Set and Test Set. Based on balanced training and test sets, a series of naive Bayesian classifiers of drug-likeness were built. As mentioned above, the number of molecules in the training sets ranges from 20,000 to 207,854, and that in the test set is 40,000 (20,000 from MDDR and 20,000 from ACD). First, using the smallest training set of 20,000 molecules, the performance of the naive Bayesian classifiers based on molecular properties and/or structural fingerprints was evaluated. The statistics of these classifiers is summarized in Table 1. It is observed that the performance of the classifier using just 21 molecular properties is not good, indicated by a low leave-one-out (LOO) cross-validated Matthews correlation coefficient C (0.277) for the training set. By adding the molecular fingerprints in training, the Bayesian classifiers can be greatly improved. The Bayesian classifiers based on molecular properties, ECFP_4, LCFP_4, ECFP_6 or LCFP_6 fingerprints, give good performance with C values larger than 0.700. Especially, the best classifier that combines molecular properties and LCFP_6 fingerprint set yields a sensitivity of 89.3%, a specificity of 86.0%, a classification accuracy of drug-like molecules of 86.4%, a classification accuracy of non-drug-like molecules of 88.9%, and a C value of 0.753 for the training set. Then, all the naive Bayesian classifiers were validated by the predictions on the test set, and the prediction accuracy for the test set is marginally worse than that for the training set (Table 1). At the same time, four better classifiers (MP+ECFP_4, MP +LCFP_4, MP+ECFP_6, MP+LCFP_6) for the training set are also give the larger C values than the others for the test set. It is pointed out that MP stands for molecular properties. The best Bayesian classifier based on molecular properties and the LCFP_6 fingerprint set can give a sensitivity of 87.7%, a specificity of 85.0%, and a C value of 0.728 for the test set.

In order to have a deeper insight into the impact of the size of training set on the performance of the classification model, the number of the molecules in the balanced training set was changed from 20,000 to 207,854, and the corresponding classifiers were built and validated. The results show that the prediction accuracy of the Bayesian classifiers was improved with the growth of the training set. The result of the Bayesian classifier based on 21 molecular properties, and the LCFP_6 fingerprint set is shown in Figure 1 as an example. When the size of the balanced training set increases from 20,000 to 140,000, the C value for the test set increases rapidly from 0.753 to 0.818. However, when the size of the training set increases from 140,000 to 207,854, the C value for the test set only increases slightly from 0.818 to 0.829. An interesting finding is that the prediction accuracy for the training set is not always better than that for the test set. When the size of the training set increases up to 180,000, the prediction accuracies on the training (C = 0.828) and test set (C = 0.825) are quite similar. Based on the above analysis, we believe that a training set of 140,000 is enough to achieve a good balance between computational efficiency and prediction accuracy.

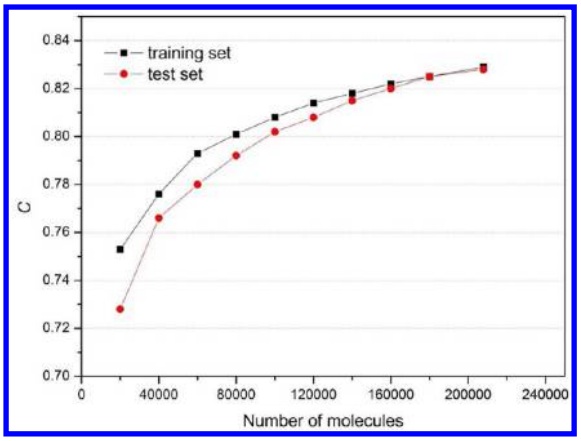


Figure 1. The Matthews correlation coefficient (C) of the naive Bayesian classifiers based on 21 molecular properties and the LCFP_6 fingerprint set by increasing the size the balanced training set from 20,000 to 207,854 molecules.

Then, using the training set of 140,000 molecules, the Bayesian classifiers based on 21 molecular properties and different fingerprint sets were trained and validated by the same test set. The *C* values for these Bayesian classifiers are shown in Figure 2. We observed that the impact of the different

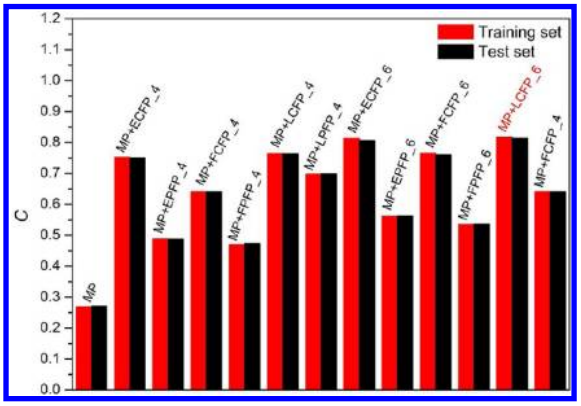


Figure 2. The Matthews correlation coefficient (*C*) of the naive Bayesian classifiers based on 21 molecular physicochemical and different molecular fingerprints. The models with the highest *C* values are colored in red.

fingerprint sets based on the training set of 140,000 on prediction accuracy is similar to that based on the training set of 20,000 molecules. As we expect, the combination of molecular properties and the LCFP_6 fingerprint set also leads to the best Bayesian classifier. This classifier gives a sensitivity of 91.4%, a specificity of 90.4%, a prediction accuracy of drug-like molecules of 90.5%, a prediction accuracy of non-drug-like molecules of 91.3%, a cross-validated *C* value of 0.818 for the training set, and a *C* value of 0.815 for the test set.

The prediction accuracy of the naive Bayesian classifier based on 21 molecular properties and the LCFP_6 fingerprint set to discriminate drug-like from non-drug-like molecules was evaluated by two bimodal histograms of the training and test sets. As shown in Figure 3, the drug-like molecules tend to have more positive Bayesian scores, while the non-drug-like molecules tend to have more negative scores. The best split value of the Bayesian score to separate drug-like from non-drug-like is around −2.358. At the same time, we observed that there is a slightly overlapped area between around −25 and 25 for both the training and test sets. So the region between −25 and 25 can be defined as the "uncertain zone". When the Bayesian score of a molecule is located in the uncertain zone, the prediction for this molecule is not reliable. At last, the quality of the best Bayesian classifier was further characterized by a receiver operating characteristics (ROC) curve (Figure 4), and the area under the ROC curve (AUC) is 0.984 for the training set of 140,000 molecules and 0.967 for the test set of 40,000 molecules, respectively, demonstrating the prediction power of the Bayesian classifier is accurate and reliable.

2. Naive Bayesian Classifiers Based on Unbalanced Training Set and Test Set. We know a general knowledge that the number of non-drug-like molecules is significantly larger than that of drug-like molecules; that is to say, non-drug-like and drug-like molecules are quite unbalanced. In order to investigate the effect of the unbalanced composition of the training set on the prediction capability of the Bayesian classifiers, multiple unbalanced training and test sets were prepared and the corresponding classifiers were constructed

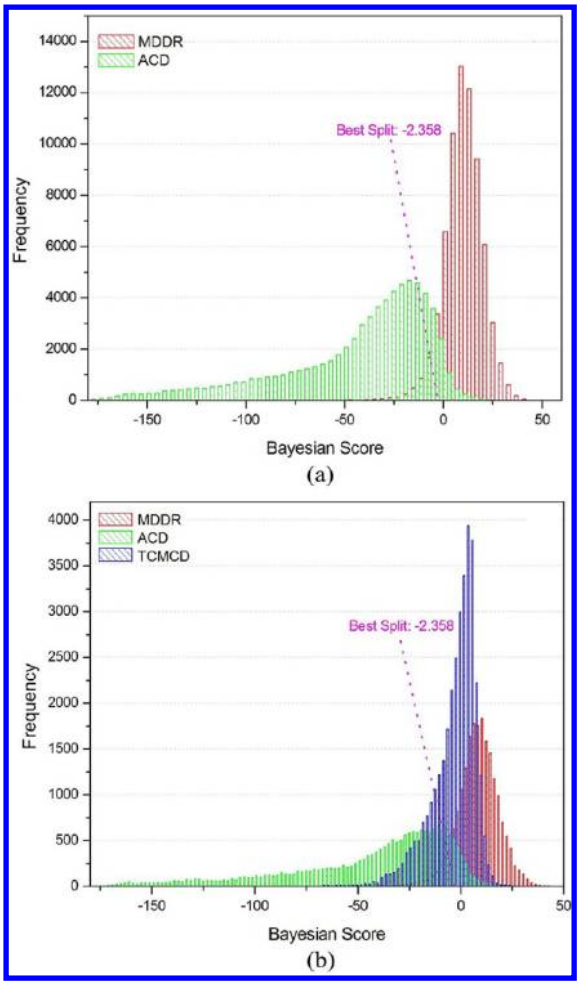


Figure 3. The distribution of the Bayesian scores predicted by the naive Bayesian classifier based on 21 molecular physicochemical properties and the LCFP_6 fingerprint set for (a) the training and (b) test sets. The Bayesian scores for the training set were obtained by using the LOO cross-validation process.

and validated. For each Bayesian classifier, the same ratio of drug-like to non-drug-like molecules was used for both training and test sets, and the ratio of drug-like to non-drug-like molecules was changed from 1:2 to 1:9. For example, if a ratio of 1:2 was used, the training set had 10,000 drug-like and 20,000 non-drug-like molecules, and the test set had 2,000 drug-like and 4,000 non-drug-like molecules. The prediction accuracies of the Bayesian models based on the unbalanced training and test sets are shown in Figure 5. Clearly, the prediction accuracy of the Bayesian model decreased with the increase of the ratio of drug-like to non-drug-like molecules. When the ratio increases from 1:2 to 1:9, the *C* values of the Bayesian model decrease from 0.726 to 0.564 for the training set and 0.739 to 0.575 for the test set. At the same time, the number of the false positives increases rapidly from 2871 to 11949 for the training set and from 545 to 2282 for the test set; however, the number of the false negatives only changes slightly. Unlike the *C* values for the balanced training sets, which increase as a function of the training set size, the *C* values for the unbalanced training sets decrease as a function of the training set size. Our observation is not surprising because the false positives increase much faster than the false negatives when the ratio of drug-like to non-drug-like molecules decreases. Obviously, the classifiers based on different training

dx.doi.org/10.1021/mp300198d | *Mol. Pharmaceutics* 2012, 9, 2875−2886

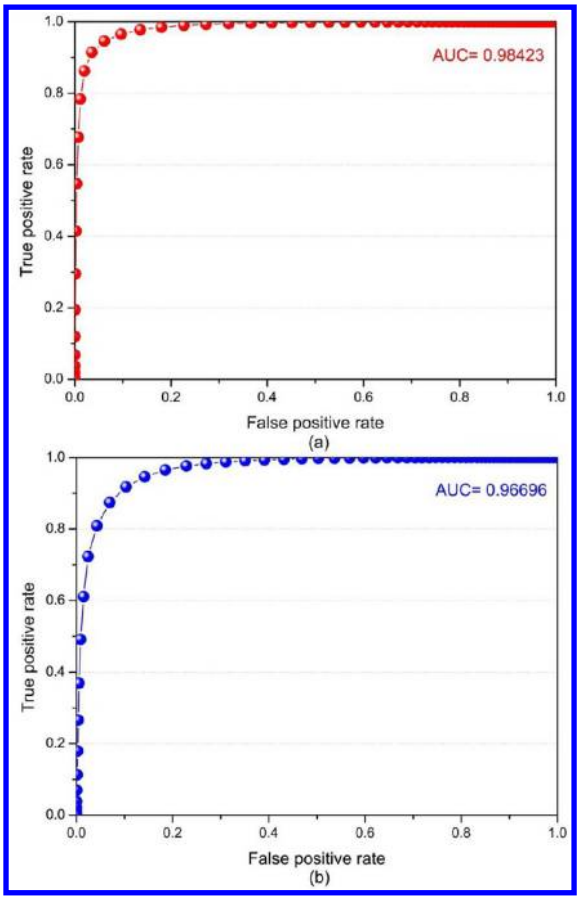


Figure 4. The ROC curves of the best Bayesian classifier for (a) the training and (b) test sets.

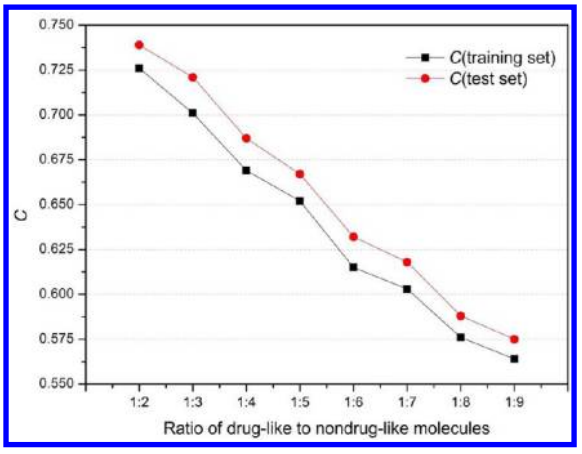


Figure 5. The Matthews correlation coefficient (C) of the naive Bayesian classifiers based on 21 molecular properties and the LCFP_6 fingerprint by decreasing the ratio of drug-like to non-drug-like molecules from 1:2 to 1:9 for (a) the training and (b) test sets.

sets with different ratios cannot be compared directly, and then it is really difficult for us to choose the best ratio. Considering the stability of the prediction, the analysis based on a classifier trained from a balanced training set may be a better choice. So in the following discussions, the Bayesian classifier based on the balanced training set of 140,000 molecules was used.

3. Analysis of the Important Fragments for Drug-likeness. According to the Bayesian classifier, the relative importance of each fingerprint is ranked by a Bayesian score, and these important molecular fragments may be useful for experimental and computational chemists to design molecules with better drug-likeness property. Based on the best Bayesian classifier trained from the balanced training set of 140,000 molecules using 21 molecular properties and the LCFP_6 fingerprint set, we got the top 6000 good and 6000 bad molecular fragments. As shown by the cumulative percentage of the molecular fragments in Figure 6, the top 2500 good or bad

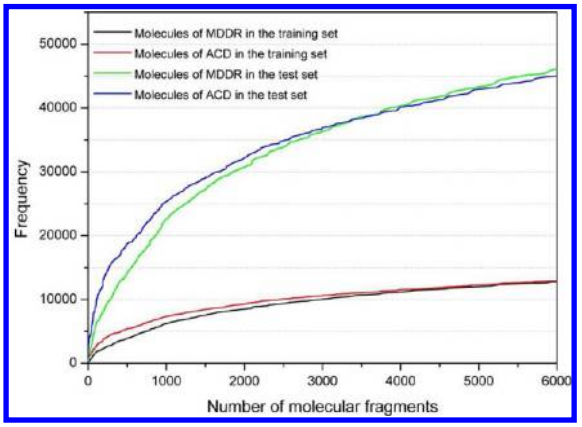


Figure 6. The cumulative percentage of the top 6000 good and 6000 bad fragments identified by the best Bayesian classifier for the molecules in the data set.

molecular fragments are shared by more than half of the molecules in the training and test sets. Therefore, the drug-like and non-drug-like molecules comprise some common chemical substructure or molecular fragments.

The top 30 good and 30 bad fragments ranked by the Bayesian scores are shown in Figure 7. These good fingerprints shown in Figure 7a give us some hints about how a molecule can be more drug-like. We systematically analyzed the pharmacological activities of the MDDR molecules in the training set that have the top 10 good fragments (Figure 8). It is interesting to observe that the molecules which have the same fragment or substructure usually have similar pharmacological activities suggested by MDDR. Specifically, the pharmacological activity for most MDDR molecules with fragment 1 is bronchodilator, those for most MDDR molecules with fragment 2 are antiarthritic and antineoplastic, that for most MDDR molecules with fragments 3, 7, and 8 is antihypertensive, those for most MDDR molecules with fragment 4 are antiarrhythmic and antianginal, and that for most MDDR molecules with fragments 5, 6, 9, and 10 is carbapenem antibiotic. As an example, the 20 molecules with the top 2 fragments are shown in Figure 9. According to our observations, we can make the following conclusion: molecules which have some key substructures identified by the Bayesian classifier are more likely to have similar or the same pharmacological activity; in other words, the important molecular fragments may be used as the structural signatures to infer the pharmacological effect of the studied molecules.

The top 30 fingerprints unfavorable to drug-likeness are shown in Figure 7b. To understand why these substructures are unfavorable is not very straightforward. One possible reason may be these substructures have reactive centers. For example, three of these fingerprints (fragments 3, 6, and 11) have the 1,2-dicarbonyl group, which is a representative reactive

dx.doi.org/10.1021/mp300198d | Mol. Pharmaceutics 2012, 9, 2875−2886

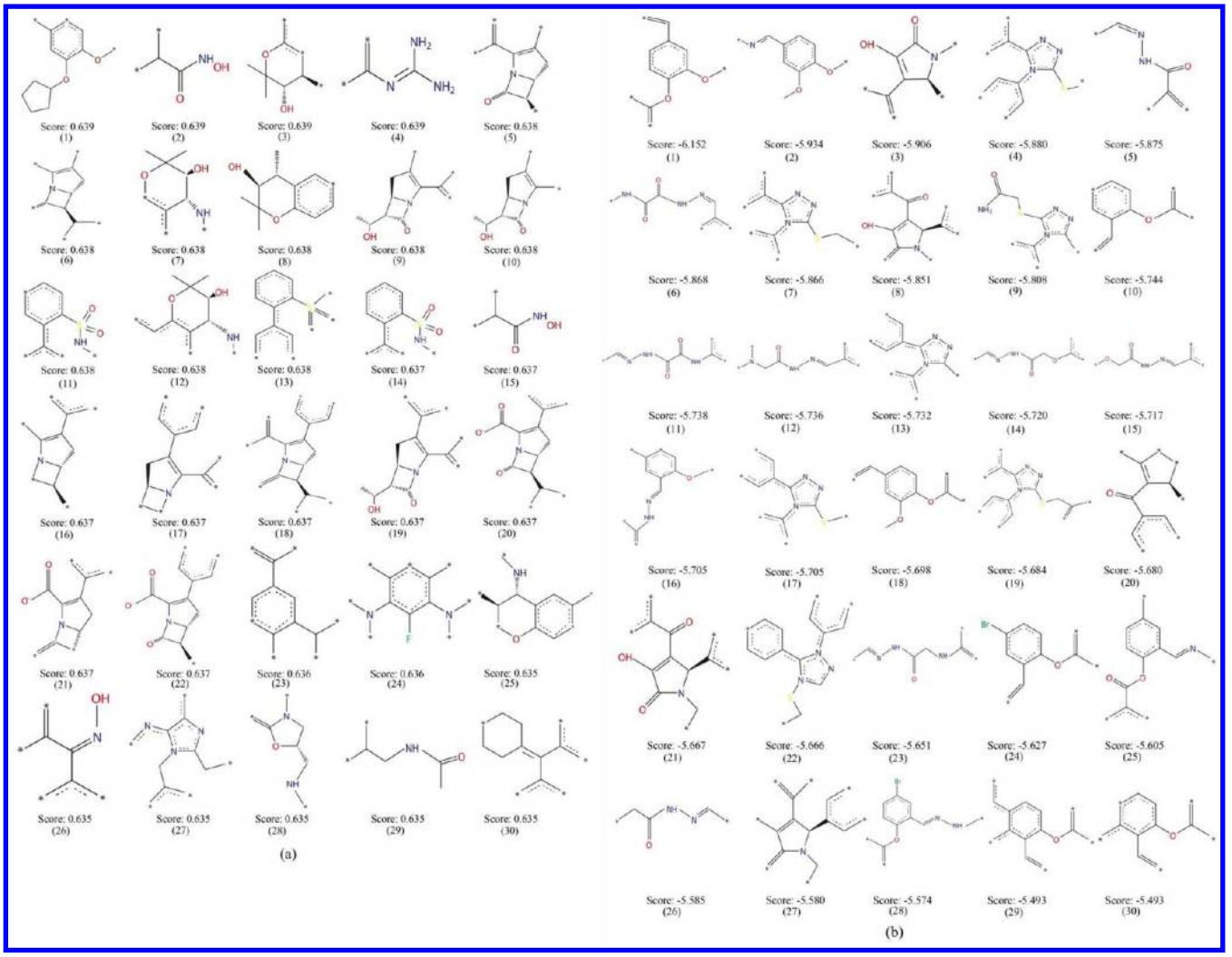


Figure 7. (a) The top 30 good and (b) 30 bad molecular fragments for drug-likeness identified by the Bayesian classifier based on 21 molecular properties and the LCFP_6 fingerprint set.

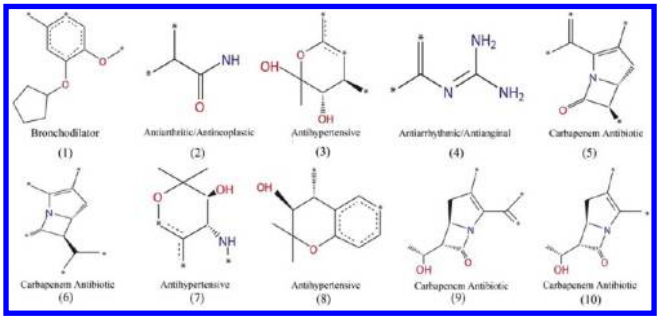


Figure 8. The primary pharmacological activities of the molecules which have the top 10 good fragments in MDDR.

functional group responsible for *in vitro* false positives of experimental binding assay.[35,36]

4. The Analysis of the Misclassified Molecules. By applying the best Bayesian classifier, 1814 drug-like MDDR molecules in the test set were predicted as false negatives and 1883 non-drug-like ACD molecules in the test set were predicted as false positives. The 20 ACD molecules with the highest probability for drug-like and 20 MDDR molecules with the highest probability for non-drug-like are shown in Figure 10.

As shown in Figure 10a, it is obvious that the top 20 non-drug-like molecules in MDDR have unfavorable fragments given by the Bayesian analysis. For example, molecules 1, 2, and 3 have fragment 5 as shown in Figure 7b. It is interesting to find that molecules 7, 8, 10, and 14 in Figure 10a contain fluorene group, which is consistent with the observation reported by an earlier work.[18] Besides, we found that four molecules in Figure 10a violate at least two rules in Lipinski's "rule-of-five". Therefore, the existence of non-drug-like molecules in MDDR was partially caused by the existence of fragments that may violate Lipinski's "rule-of-five". Then, we checked the development stage of these top 20 non-drug-like molecules in MDDR and found that 18 molecules were under biological test, and 2 were under preclinical phase. It is quite possible that these predicted non-drug-like molecules in MDDR were under the early stage of drug discovery.

Similarly, according to the Bayesian scores, the top 20 drug-like molecules in ACD in the test set are shown in Figure 10b. The top 20 false positives from ACD probably possess favorable fragments for drug-likeness. For example, molecules 1, 2, 3, 13, 14, 15, and 19 contain fragment 29 shown in Figure 7a. There is no completely non-drug-like database available to date. Although ACD is usually used as a non-drug-like data set, some ACD compounds are also used in high-throughput

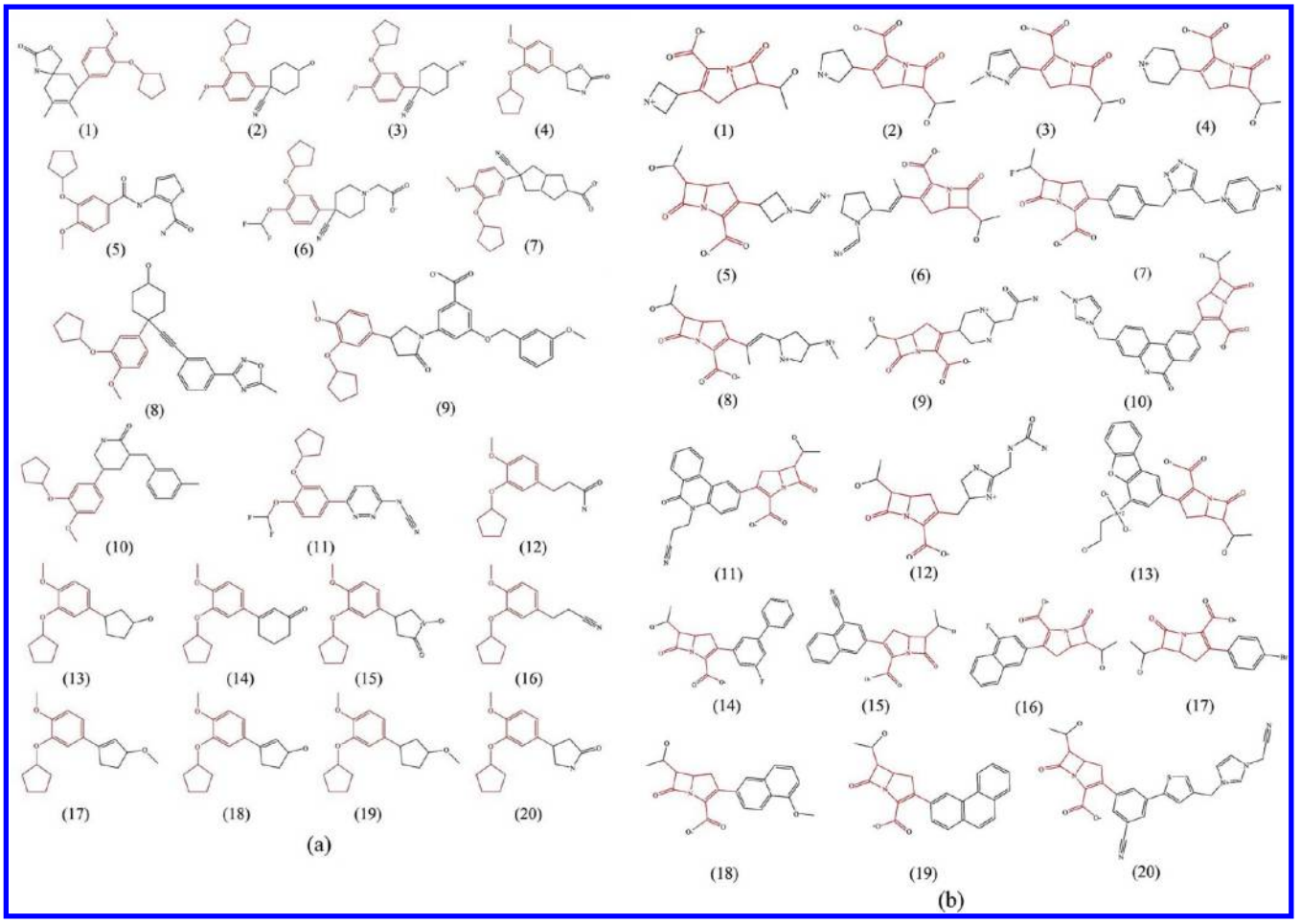


Figure 9. (a) The 20 molecules which have the best fragment and (b) 20 molecules which have the top 2 fragments found in MDDR.

screenings. Therefore, it is not surprising that ACD contains many drug-like molecules. Then, we checked the molecular similarity between the top 20 drug-like molecules in ACD and the 70000 molecules in MDDR in the training set. For these 20 false positives, 846 similar drug-like molecules could be found in MDDR. This may be an important reason for the misclassification of these 20 ACD molecules. According to our predictions, about 9% of the compounds in ACD are drug-like molecules, and these drug-like molecules in ACD predicted by the Bayesian classifier need more attention in virtual screening.

5. Recursive Partitioning Classifiers of Drug-likeness. Compared with the Bayesian classification technique, RP can give simpler and more understandable classification models described by a series of hierarchical rules. Therefore, RP was used to establish decision trees to classify molecules into drug-like and non-drug-like categories, and we tried to compare the performance of the Bayesian and RP classifiers based on the same descriptors and the same balanced training set. In the RP analysis, a very important parameter to control the complexity of a decision tree, the tree depth, was calibrated carefully according to the predictions on the test set. The tree depth was changed from 5 to 18, and the prediction accuracies of the corresponding models were evaluated. The change of the C value versus the tree depth is shown in Figure 11. For the training set, the C value increases with the increase of the tree depth; whereas for the test set, the C value does not always increase along with the increase of the tree depth. For the test set, the value of C increases quickly from tree depth 5 to 12, and then increases slightly and reaches the maximum value at tree depth 13 (C = 0.525). At the same time, when the tree depth is larger than 13, the C value for the test set does not increase any more and even decreases slightly. At last, the best RP classifier was trained at the tree depth of 13. The best RP model gives a sensitivity of 80.1%, a specificity of 72.2%, a classification accuracy of 74.2% for the drug-like class, a classification accuracy of 78.4% for the non-drug-like class, and C of 0.525 for the test set. Based on the same descriptors and data set, the naive Bayesian classifier is clearly much better than the RP classifier.

6. Is TCMCD Drug-like or Non-Drug-like? As we mentioned above, we already obtained a Bayesian classifier of drug-likeness with impressive prediction accuracy based on a balanced training set of 140,000 molecules. We then used this Bayesian classifier to evaluate the drug-likeness of the molecules in TCMCD. It should be noted that a subset of TCMCD with 33,961 molecules (molecular weight is less than 600) was extracted, and the molecular weight distribution of the TCMCD subset is similar to that of the MDDR subset for training. We expect that the quantitative predictions can solve a puzzle that has fascinated medicinal and computational chemists for years: is TCMCD drug-like or non-drug-like? According to our predictions, in the TCMCD subset, 20,163 were classified as drug-like molecules and the others as non-drug-like molecules, and the percentage of the drug-like molecules in TCMCD is 59.37%. If all molecules in TCMCD

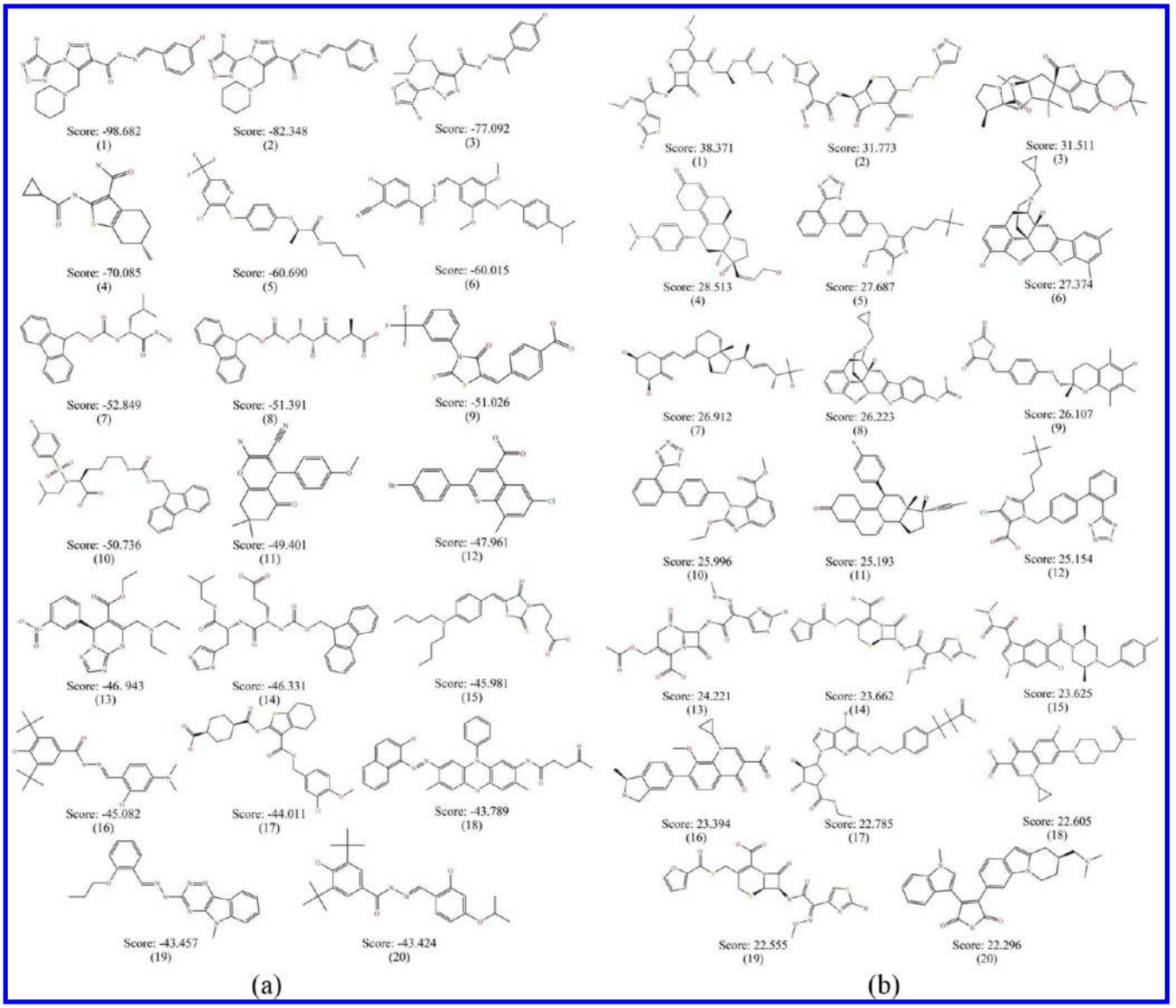


Figure 10. (a) The 20 MDDR molecules with the highest probability for non-drug-like and (b) 20 ACD molecules with the highest probability for drug-like.

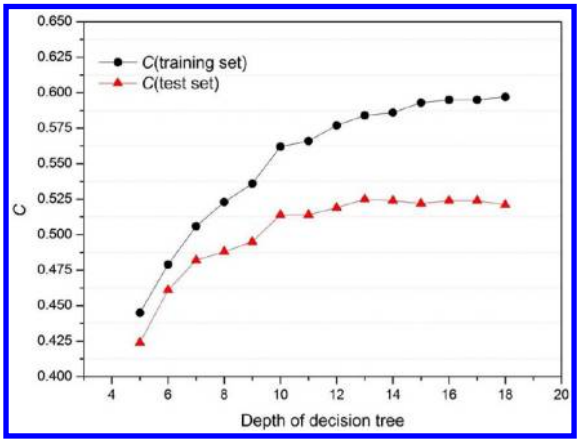


Figure 11. The change of *C versus* the tree depth for (a) the training and (b) test sets. The RP model was constructed based on 21 molecular physicochemical properties and the LCFP_6 fingerprint set.

were included in the drug-likeness analysis, 43,108 molecules (67.61%) were predicted to be drug-like. That is to say, TCMCD is more drug-like than non-drug-like. Newman and co-workers pointed out that half of new chemical entities (NCEs) approved by the FDA were of natural product origin or derived from natural products in the period of 1981−2002.[37] Certainly, the molecules in TCMCD are important components of natural products. Therefore, we believe that TCMCD is a good resource of drug-like molecules.

In order to understand the distributions of some important molecular fingerprints in TCMCD, we investigated the molecules in TCMCD which contain the top 50 fragments. Twenty molecules from TCMCD that have the top 50 fingerprints favorable for drug-likeness are shown in Figure 12. Moreover, the primary pharmacological activities of these fingerprints are also shown in Figure 12. Our analysis and extensive literature searching show that the biological activities of some molecules in Figure 12 have been reported. For example, molecule 2 has a cardioprotective effect,[38,39] molecule 3 has cardiotonic and anticonvulsant activities,[40,41] molecule 7

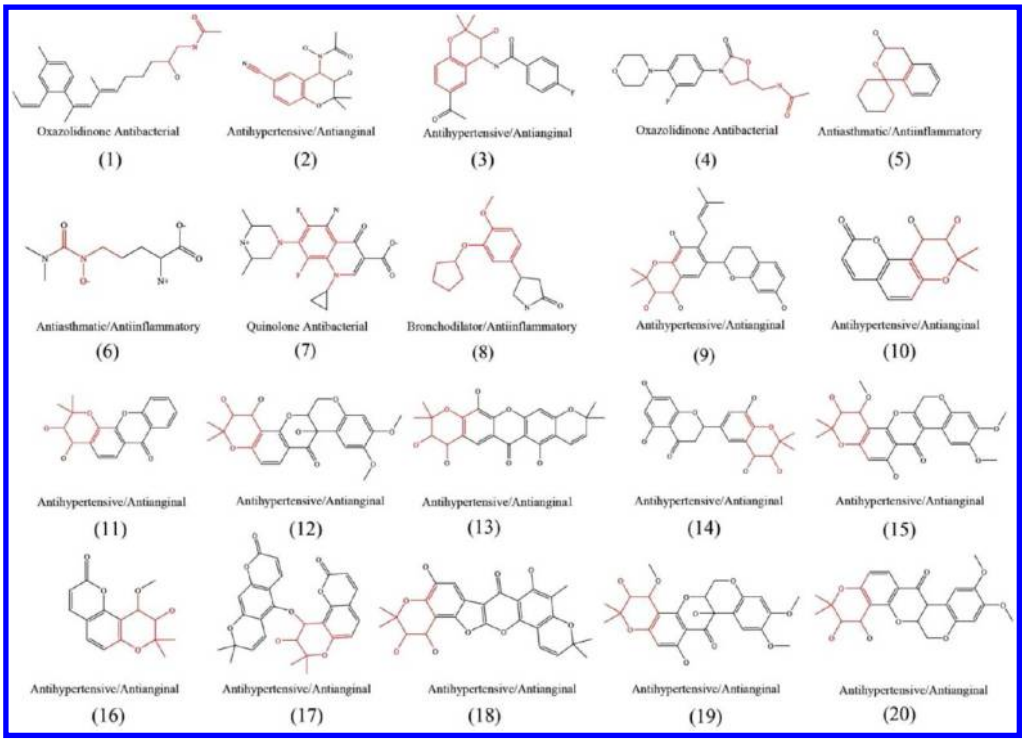


Figure 12. The pharmacological activities of 20 molecules from TCMCD that have the top 50 good fragments.

has antibacterial and antibiotic activities,[42−45] molecule 8 shows anti-inflammatory, vasodilation, and phosphodiesterase (PDE) III and IV inhibiting activities,[46−49] and molecules 11, 12, 13, and 20 exhibit antibacterial and antifungal effects.[50,51] It is exciting to find that the experimental pharmaceutical activities of four molecules (2, 3, 7, and 8) are the same as or quite similar to those predicted by the pharmaceutical effect of the fingerprints. For example, the activities of molecule 8 that contains the fragment with the highest Bayesian score are quite similar to those (anti-inflammatory, bronchodilator, and phosphodiesterase (PDE) IV activities) of the molecules in MDDR that contain the same fragment (Figure 7a). Therefore, the molecules in TCMCD and those in MDDR that share the same important fingerprints may have similar or even the same bioactivities.

We know that pharmacological research on TCMCD at the enzyme or cell level is still limited and the pharmacological activities for most molecules in TCMCD are unknown. It may be a good way to use the pharmacological effects of the important fingerprints to annotate the pharmacological activities of the molecules in TCMCD. We hope that this study can give some clues to help medicinal and computational chemists speed up the development of TCMs.

CONCLUSIONS

In this work, based on the MDDR and ACD subsets with similar molecular weight distributions, the prediction models of drug-likeness were developed by the naive Bayesian classification and recursive partitioning techniques. First, the impact of molecular properties and different fingerprint sets on prediction was investigated systematically, and we found that the addition of molecular fingerprints could improve the prediction significantly, especially the LCFP_6 fingerprint set. Moreover, a variety of Bayesian classifiers were constructed by changing the ratio of drug-like to non-drug-like molecules and the size of the training set. The results indicate that the prediction accuracy of the Bayesian classifiers is closely related to the size and the degree of balance of the training set. When a balanced training set was used, the best Bayesian classifier based on 21 molecular physicochemical properties and the LCFP_6 fingerprint set yielded an LOO cross-validated accuracy of 91.4% for the training set and 90.9% for the test set. On the other hand, most important molecular fragments favorable or unfavorable for drug-likeness were identified by the Bayesian analysis, and they would be very helpful to design high quality lead compounds in the early stage of the drug design/discovery process. Finally, the drug-likeness of TCMCD was evaluated by the best Bayesian classifier, and we found that most molecules in TCMCD are drug-like. Based on this observation, TCMCD was believed to be a good source of drug-like molecules. We hope that our study can stimulate the development of TCMs in the future.

ASSOCIATED CONTENT

Supporting Information

Part 1 providing the protocol to preprocess the three datasets. Table S1 providing the descriptions of the 21 molecular descriptors used for drug-likeness analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by the National Science Foundation of China (20973121 and 21173156 to T.H.), the National Basic Research Program of China (973 program, 2012CB932600), the NIH (R21GM097617 to J.W.), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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