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Development of novel *in silico* prediction model for drug-induced ototoxicity by using naïve Bayes classifier approach

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ABSTRACT

Some drugs have the potential to cause cellular degeneration of cochlear and/or vestibular system, leading to temporary or permanent hearing loss, innitus, ataxia, dizziness, ear infections, hyperacusis, vertigo, nystagmus and other ear problems. Thus, precise assessment of ototoxicity has become a strong urge task for the toxicologist. In this research, the *in silico* prediction model of ototoxicity was developed based on 2612 diverse chemicals by using naïve Bayes classifier approach. A set of 7 molecular descriptors considered as important for ototoxicity was selected by genetic algorithm method, and some structural alerts for ototoxicity were identified. The established naïve Bayes prediction model produced 90.2% overall prediction accuracy for the training set and 88.7% for the external test set. We hope the established naïve Bayes prediction model should be employed as precise and convenient computational tool for assessing and screening the chemical-induced ototoxicity in drug development, and these important information of ototoxic chemical structures could provide theoretical guidance for hit and lead optimization in drug design.

1. Introduction

Some drugs currently in clinical use can cause cellular degeneration of cochlear and/or vestibular system, leading to temporary or permanent hearing loss, innitus, ataxia, dizziness, ear infections, hyperacusis, vertigo, nystagmus and other ear problems (Arslan et al., 1999; Bisht and Bist, 2011; Cianfrone et al., 2011; Sedó-Cabezón et al., 2014). Such as, aminoglycoside antibiotics could damage the cochlea and vestibular apparatus, leading to irreversible hear loss (Buszman et al., 2003; Chen et al., 2007; Van Boeckel et al., 2014). Platinum-based chemotherapeutic agents have been reported to cause cell death in outer hair cells, and subsequently resulting in sensor ineural hear loss (Rybak et al., 2007, 2000). Other agents, such as loop diuretics, macrolide, quinine and antimalarials, have been proven to have ototoxic potential (Campo et al., 2013; Chiodo and Alberti, 1994; Ding et al., 2016; Schacht et al., 2012). One strategy to prevent the ear from ototoxicity of chemicals is that the precise assessment of the ototoxicity of drug candidates in early research and development (R&D) stage. However, the conventional *in vivo* animal auditory functional methods and *in vitro* histological methods are costly and labor intensive (Cunningham, 2006; Matsui et al., 2004; Poirrier et al., 2010; Yorgason et al., 2011), which may not be suitable for screening large numbers of samples in early drug development. Therefore, development of simple, rapid and convenient methods for assessment of ototoxicity has become imperative for the toxicologist. Compared with experimental approaches, the computational methods have advantages of cost-effectiveness and faster to generate results. Thus, developing convenient *in silico* methods to evaluate the ototoxicity of chemicals is a strong urge task in drug development.

Presently, employing computational techniques for different types of toxicities assessment has been explicitly encouraged by some international regulatory agencies (OECD, 2007; REACH, 2011). However, compared with other toxicological end points, using *in silico* models for ototoxicity prediction was relatively fewer. To the best of our knowledge, only Zhou et al. (2014) established support vector machine (SVM) prediction models for drug-induced ototoxicity based on 32 molecular descriptors, and the best prediction model gave 85.33% and 83.05% for two independent test sets, respectively. Thus, developing more precise and accurate computational models for drug-induced ototoxicity prediction with using larger data is very necessary. In addition, investigating structure characteristics of ototoxic chemical is also vital, which may contribute to the explanation of the mechanisms of ototoxicity, and further provide theoretical guidance for hit and lead optimization in drug design. Therefore, in this study, another classical

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computational method, naïve Bayes classifier, was considered to assess drug-induced ototoxicity. The naïve Bayes classifier, considered as one of the most effective machine learning algorithms based on the Bayes' theorem with conditional independence assumptions (Berger, 2013; Box and Tiao, 2011). It performs surprisingly well and has been widely applied to various toxicities prediction (Zhang et al., 2017).

In this research, a larger ototoxicity data set with 2612 compounds was applied to develop a novel prediction model using naïve Bayes method. The genetic algorithm method was used to find the important molecular descriptors associated to ototoxicity, and the ECFP_14 fingerprints were employed for the ototoxic/non-toxic fragments production. The classical internal 5-fold cross validation and external test set were used to validate the established prediction models. Furthermore, the important descriptors for ototoxicity prediction and ototoxic/non-toxic fragments produced by the ECFP_14 were carefully analyzed. Finally, the structural features of these misclassified compounds in the external test set were also carefully analyzed. We hope the established naïve Bayes prediction model could be used as a prediction and screening tool of potential ototoxicity in drug development, and the important information of ototoxic chemical structures obtained in this research could provide theoretical guidance for hit and lead optimization in drug design.

2. Materials and methods

2.1. Dataset preparation

The 897 pharmaceutical agents with ototoxical potential, including drugs, medications, chemicals and herbals, were extracted from the reference that contained the most updated collections of ototoxic agents (Bauman, 2010). Compared with these ototoxic compounds, few of literature resources clearly declared that a drug was negative for ototoxicity. Therefore, to guarantee the collected agents are real negative for ototoxicity, some drugs that have been widely used in clinical and have no any report about their potential ototoxicity risk were selected as negatives. Then, 1715 drugs were selected and considered as negative for ototoxicity. Finally, a total of 2612 compounds, including 897 ototoxicans and 1715 non-toxicants, were used in this study. The dataset was then randomly divided as training set with 2162 compounds, (83%) and test set with 450 compounds (17%) by the "Generate Training and Test Data" protocol in Discovery Studio 3.5 (Table 1). The list of all chemicals, including their chemical structures and categories, were given in the supplementary material. The work flow of the predictive modeling for drug-induced ototoxicity was displayed in Fig. 1.

2.2. Molecular descriptors calculation

In this research, the categories of element counts, AlogP, molecular properties, molecular property counts, surface area and volume and topological descriptors were calculated by using the Discovery Studio 3.5. 140 molecular descriptors were finally obtained. Then, we preprocessed these descriptors, such as descriptors with all zero values or zero variance and correlation between all pairs was higher than 0.95 were removed. Finally, 67 descriptors were remained, and further selected by using genetic algorithm.

In addition, the topological fingerprints descriptors were considered and applied in this study (Valdés-Martiní et al., 2017). Among these topological fingerprints, the extended-connectivity fingerprints (ECFPs) have been widely employed for structure-activity modeling, similarity searching, clustering, and virtual screening (Morgan, 1965; Rogers and Hahn, 2010). Moreover, the extended-connectivity fingerprints (ECFPs) could significantly influence the prediction performance of classification models (Zhang et al., 2017). In this investigation, the ECFP_14 fingerprints were used to generate the structural features of ototoxic/non-toxic compounds because it significantly influences the prediction performance.

Table 1
The number of ototoxicants and non-toxicants applied in the training set and test set.

	Training set	Test set	Sum
Ototoxicants	782	115	897 (34%)
Non-toxicants	1380	335	1715 (66%)
Total	2162 (83%)	450 (17%)	2612 (100%)

2.3. Feature selection with genetic algorithm strategy

Genetic algorithm (GA) is inspired by evolution theory, which has been widely applied to feature selection (Davis, 1991). In genetic algorithms, the chromosomes are represented by a set of features, which compose a population. The GA starts with initial population, and a new population is created with using some operators: selection, mutation and crossover. GA repeatedly generates population, and then evaluates their fitness. In this study, the binary string with each bit representing a feature was used to represent a chromosome (0: not selected, 1: selected). The GA was carried out in the gcc programming language, in which the roulette wheel selection algorithm was used to choose the chromosomes for crossover to produce offspring, the crossover rate was set to 0.5, and the mutation rate was defined as 0.05. The algorithm terminated when reaching 100 iterations. The time for running 100 iterations generally takes about 8 to 12 h.

2.4. Naïve Bayes (NB) classifier

The naïve Bayes classifier method is a simple probabilistic classifier that assigns each object to the class with strong independence assumptions between variables. In our study, each compound can be categorized into ototoxicant class (+) or non-toxicant class (−), the class variables is represented by the "c". the variables (molecular descriptors and fingerprints) are defined as f_1, f_2, …, f_n, the vector is described as $F = (f_1, f_2, \ldots, f_n)$.Then Bayes' theorem describes as:

$$P(c\ |F) = \frac{P(F\ |c)P(c)}{P(F)} \quad (1)$$

where $P(c)$ represents the prior probability, $P(F)$ is the marginal probability, $P(c|F)$is the posterior probability of the compound class, $P(F|c)$ indicates the conditional probability, respectively. In our study, the naïve Bayes classifiers were developed by using Discovery Studio (DS) version 3.5 (http://accelrys.com/products/discovery-studio/).

2.5. Recursive partitioning (RP) classifier

The recursive partitioning (RP) is a statistical method, which could create one or more decision trees to describe the relationship between a dependent property Y (*e.g.*, toxicity class) and a set of independent properties X (*e.g.*, molecular descriptors and fingerprints) (Strobl et al., 2009). It classifies members of the population based on a dichotomous splitting a data set into smaller and smaller subsets. RP has become popular and widely used tools for prediction of ADME/Tox Properties. In this investigation, the "Split Method" was selected as Gini and the "Weighting Method" was set as Uniform. The RP classification model was developed using the Discovery Studio (DS) version 3.5 (http://accelrys.com/products/discovery-studio/), the minimum number of samples at each node was changed from 2 to 10, and the maximum tree depth was changed from 2 to 30 systematically in order to find a better RP model.

2.6. Evaluation and validation of the classification models

In this research, the reliability and predictive ability of the

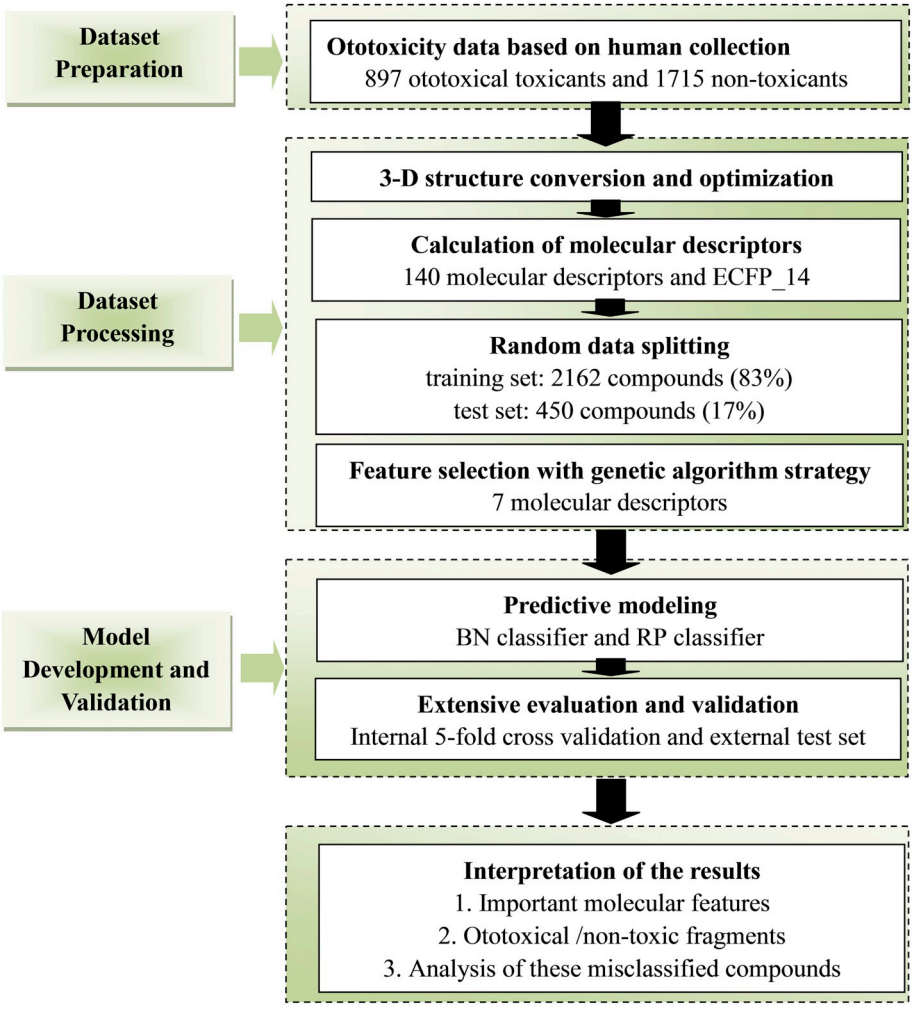


Fig. 1. The work flow of the predictive modeling for drug-induced ototoxicity.

Table 2
The parameters used in this study to measure the predictive performance of the classification models.

Parameters	Abbreviation	Equations
Overall prediction accuracy	Q	$Q = \frac{TP + TN}{TP + TN + FP + FN} \times 100\%$
Sensitivity	SE	$SE = \frac{TP}{TP + FN} \times 100\%$
Specificity	SP	$SP = \frac{TN}{TN + FP} \times 100\%$
Positive predictive value	PPV	$PPV = \frac{TP}{TP + FP} \times 100\%$
Negative predictive value	NPV	$NPV = \frac{TN}{TN + FN} \times 100\%$
Balanced accuracy	BA	$BA = \left[\left(\frac{TN}{TN+FP} + \frac{TP}{TP+FN}\right)/2\right] \times 100\%$
Matthews Correlation Coefficient	MCC	$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP+FP)\times(TP+FN)\times(TN+FP)\times(TN+FN)}} \times 100\%$

classification models were assessed by the internal 5-fold cross validation and an external independent test set. The following parameters, based on true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN), were used (Table 2): the overall prediction accuracy (Q), sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), balanced accuracy (BA), and Matthews Correlation Coefficient (MCC). Among these parameters, the Matthews Correlation Coefficient (MCC) is used to measure the quality of binary machine learning classifications, which value ranges from −1 to 1, and − 1 indicates a completely wrong binary classifier while 1 indicates a perfect classification.

3. Results

3.1. Selection of the important descriptors for ototoxicity

Feature selection is very important for classification modeling. In this research, 140 molecular descriptors were initially calculated. After elimination of those redundancy and overlapping descriptors, 67 descriptors were remained. Then, the genetic algorithm was employed for selection of molecular features carrying information about ototoxicity. Finally, the 7 molecular descriptors, including O_count, Log D, Num_H_Donors, Num_Rings 6, Num_Aromatic Rings, IAC_Total and

Table 3
The molecular descriptors selected by genetic algorithm and their Cramer's V Coefficient (φ_c) to ototoxicity.

Descriptor	φ_c	Explanation
O_count	0.228	Number of oxygen atoms
Log D	0.218	The octanol–water partition coefficient calculated taking into account the ionization
Num_H_Donors	0.227	Number of hydrogen bond donors
Num_Rings 6	0.144	Number of rings of size 6
Num_Aromatic Rings	0.141	Number of aromatic rings
IAC_Total	0.365	Graph-theoretical info content descriptors
SC_0	0.322	Sub graph counts

SC_0, were selected, which were considered as the most important descriptors associated with ototoxicity. The explanations for these 7 selected descriptors were given in Table 3.

3.2. Construction of the prediction model for ototoxicity by using naïve bayes classifier

The 2162 agents, comprising of 782 ototoxic compounds and 1380 non-toxicants, were used as the training set to construct the naïve Bayes prediction model of ototoxicity. The naïve Bayes prediction model was successfully developed based on the 7 molecular descriptors and ECFP_14 fingerprints (NB-1), in which the parameter of "number of bins" was set as 50. For the training set, the 5-fold cross validation was used as the internal validation to evaluate the reliability of the naïve Bayes prediction model. The prediction results of the training set were displayed in Table 4. As can be seen from the Table 4, the established naïve Bayes prediction model (NB-1) produced 90.2% overall prediction accuracy (*Q*), 88.7% balanced accuracy (BA), 83.4% sensitivity (SE), 94.0% specificity (SP), 88.7% positive predictive value (PPV) and 90.9% negative predictive value (NPV), respectively. The MCC of the training set was 0.785. Obviously, the prediction results of the training set indicated that the established naïve Bayes model (NB-1) had good discrimination ability in ototoxicity prediction.

3.3. Validation of the established naïve bayes model by an independent test set

Except for internal validation, the external validation was recognized as the most rigorous and true test of the predictive power of a QSAR model. In this investigation, the 450 agents, comprising of 115 ototoxic chemicals and 335 non- ototoxic agents, were used as external test set to measure the predictive power of the naïve Bayes classification model (NB-1). The statistical results for the external test set were listed in Table 4. The established naïve Bayes classification model (NB-1) gave sensitivity (SE) of 84.3 %, specificity (SP) of 90.1%, positive predictive value (PPV) of 74.6%, negative predictive value (NPV) of 94.4%, balanced accuracy (BA) of 87.3% and overall prediction accuracy (*Q*) of 88.7%. The MCC of the test set was 0.717. Obviously, the established naïve Bayes model (NB-1) presented a good predictive performance for the ototoxicity prediction, and could correctly discriminate external agents as ototoxic chemicals and non-toxicants with using 7 molecular descriptors and ECFP_14 fingerprints.

Table 4
The detailed prediction results for the training sets and test sets given by naïve Bayes classifier (NB) and recursive partitioning classifier (RP).

Model name		SE	SP	PPV	NPV	*BA*	*Q*	MCC
NB-1	Training	83.4	94.0	88.7	90.9	88.7	90.2	0.785
	test	84.3	90.1	74.6	94.4	87.3	88.7	0.717
NB-2	Training	66.2	59.8	48.2	75.8	63.0	62.1	0.250
	test	71.3	62.4	39.4	86.4	66.9	64.7	0.295
RP	Training	49.2	91.6	76.9	76.1	70.4	76.3	0.465
	test	64.4	88.1	64.9	87.8	76.2	82.0	0.526

4. Discussions

4.1. Analysis of the important descriptors for chemical-induced ototoxicity

The 7 molecular descriptors (O_count, Log D, Num_H_Donors, Num_Rings 6, Num_Aromatic Rings, IAC_Total and SC_0) selected by the genetic algorithm were considered as important for ototoxicity prediction. Among these 7 simple descriptors, the O_count, Num_Rings 6 and Num_Aromatic Rings characterize the molecular property counts. The Num_H_Donors measures the hydrogen-bonding property. The Log D, as a physicochemical descriptor, estimates the octanol water distribution coefficient. The topological descriptors of IAC_Total and SC_0 describe the molecular graph information. The Cramer's V Coefficient (φ_c) is used to measure the association between two variables. The Cramer's V Coefficient (φ_c) is ranged from 0 to 1, the value of 1 indicates the two variables are completely related, and 0 indicates the two variables are unrelated. The Cramer's V Coefficient (φ_c) between descriptors and ototoxicity were calculated (Table 3). As shown in Table 3, the selected descriptors are related to ototoxicity. In addition, the assumption of normality for the two compared samples was analyzed. Because the data do not follow a normal distribution, the Pearson's chi-squared test was used to determine the statistically significant difference in the distribution of 7 molecular descriptors between ototoxicants and non-toxicants. The *p*-value was used in statistical hypothesis testing to quantify the statistical significance of sample. As can be seen from Fig. 2, the *p*-values for these 7 descriptors were less than 0.01, indicating that the distributions of molecular descriptors in ototoxicants and non-toxicants are significantly different.

The average values of the 7 descriptors for 897 ototoxicants and 1715 non-toxicants are also given in Table 5. As can be seen from the Fig. 2 and Table 5, the number of O atoms descriptor is distributed between 0 and 18 with a mean of 3.14 for the ototoxicants and between 0 and 8 with a mean of 2.38 for the non-toxicants, respectively. The logD is ranged from −12.47 to 9.57 with a mean of 1.29 for the ototoxicants and from −8.44 to 8.24 with a mean of 1.52 for the non-toxicants, demonstrating that ototoxicants usually have higher hydrophobicity than that of non-toxicants. For the number of H donors, the toxic class is distributed between 0 and 15 with a mean of 1.92, and the non-toxic class is distributed from 0 to 5 with a mean of 1.25, suggesting that ototoxicants usually tend to be more hydrogen-bonding than that of non-toxicants. The mean value of the number of rings 6 for the ototoxicants is 1.95 and 1.62 for the non-toxicants, which is positively contributed to the ototoxicity. The mean values of number of aromatic rings for ototoxicants and non-toxicants are 1.41 and 1.16, which suggests that the ototoxicants usually contain more aromatic rings. In addition, the mean values of topological descriptor IAC_Total for ototoxicants and non-toxicants are 64.65 and 54.24, respectively. For the SC-0 descriptor, the mean value is 22.86 for the ototoxicants and 18.52 for the non-toxicants.

4.2. Analysis of the ototoxic/non-toxic fragments produced by the ECFP_14 fingerprint descriptors

The extended-connectivity fingerprints (ECFPs) are critical topological fingerprints, which initially designed for the molecular characterization, similarity searching, and structure-activity modeling. Previous researches suggested that the ECFPs significantly influenced the predictive performances of QSAR prediction models. In this investigation, in order to evaluate the ECFP_14 fingerprints influence the predictive performance of the naïve Bayes prediction model for chemical-induced ototoxicity, the NB-2 prediction model was constructed, in which only the 7 descriptors (*e.g.*, O_count, Num_H_Donors, Num_Rings 6, Num_Aromatic Rings, IAC_Total and SC_0) were applied. The detailed prediction results of the training set and test set for NB-2

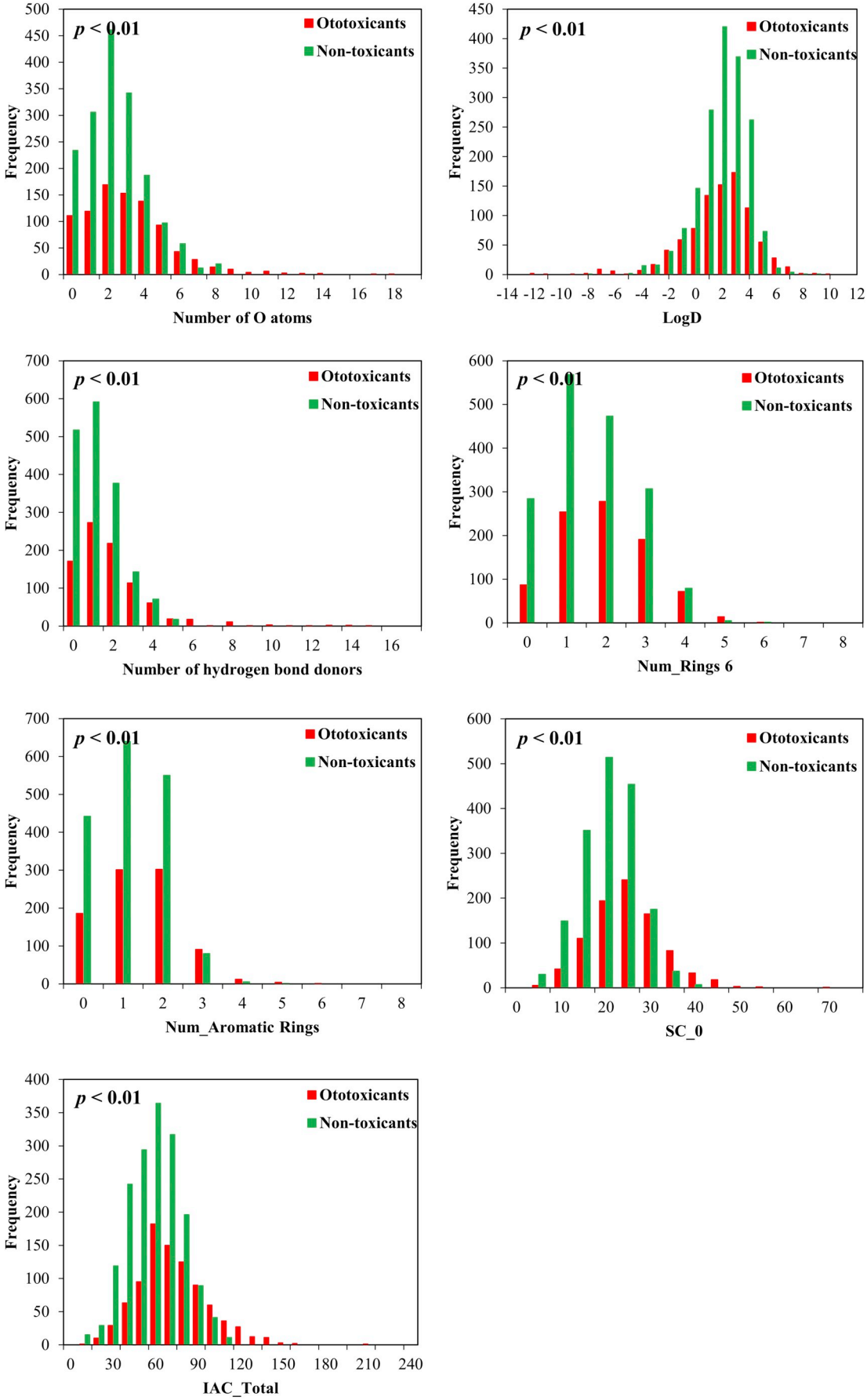


Fig. 2. Distributions of the 7 molecular physicochemical properties, including number of O_count, Log D, number of H donors, number of rings 6, number of aromatic rings, IAC_total and SC_0 for the ototoxicants and non-toxicants.

Table 5
Mean physicochemical properties for the 897 ototoxic chemcials (positive molecules) and 1715 non-toxicants (negative molecules). Molecular descriptors generated in Discovery Studio 3.5.

Descriptors	Toxicants (mean ± SE[a])	Non-toxicants (mean ± SE[a])
O_count	3.14 ± 0.08	2.38 ± 0.04
Log D	1.29 ± 0.09	1.52 ± 0.04
Num_H_Donors	1.92 ± 0.06	1.25 ± 0.03
Num_Rings6	1.95 ± 0.04	1.62 ± 0.03
Num_AromaticRings	1.40 ± 0.03	1.16 ± 0.02
IAC_Total	64.65 ± 0.83	54.24 ± 0.44
SC-0	22.86 ± 0.27	18.52 ± 0.15

[a] SE, the standard error.

were presented in Table 4. For the training set of the NB-2 model, the sensitivity (SE) was 66.2%, specificity (SP) was 59.8%, positive predictive value (PPV) was 48.8%, negative predictive value (NPV) was 75.8%, balance accuracy (BA) was 63.0% and overall prediction accuracy (*Q*) was 62.1%. The MCC value was 0.250. For the test set, the NB-2 model gave 71.3% sensitivity (SE), 62.4% specificity (SP), 39.4% positive predictive value (PPV), 86.4% negative predictive value (NPV), 66.9% balance accuracy (BA) and 64.7% overall prediction accuracy (*Q*). The MCC value of the test set for NB-2 model was 0.295. Compared the prediction results produced by NB-1 and NB-2, it was found that the prediction accuracies given by NB-2 without using the ECFP_14 fingerprints significantly reduced. In addition, previous studies suggested that ECFPs fingerprints significantly influenced the prediction accuracy of the positives (sensitivity, SE). However, in this research, we found the prediction accuracies of ototoxicants (sensitivity, SE) and non-toxicants (specificity, SP) significantly improved when addition of ECFP_14 fingerprints, which demonstrated that the fragments generated by ECFP_14 significantly contributed to both ototoxicants and non-toxicants identification.

The molecular fragments produced by the ECFP_14 fingerprints were displayed in Fig. 3. The top 20 ototoxic substructures were shown in Fig. 3a. The top 20 non-toxic substructures were presented in Fig. 3b. These ototoxic and non-toxic substructures ranked by the Bayesian scores that is a measurement of how different this is from the hit rate as a whole (the ratio that would be expected if the feature was occurring randomly across the ototoxicants and non-toxicants). Carefully compared these fragments, it was obviously observed that there was no common substructure presented in the ototoxic features (Fig. 3a) and non-ototoxic features (Fig. 3b). Further analysis of these 20 ototoxic fragments displayed in Fig. 3a, some representative toxic substructures were found to be important for ototoxicants identification, such as fragments containing *N*, *N*,3-trimethylbut-3-en-2-amine group (G1, G4), dimethoxymethane (G2, G7, G11), fluorocyclohexane (G3, G6), 2-methoxycyclohexanamine (G5, G16, G20), 2-isopropoxypropane (G8), *N*-methylpropan-2-amine (G8), *N*-methylpropan-2-amine (G9), *N*, *N*-dimethylisobutyramide (G10), 2,3,5-trimthylhexa-1,3-diene (G14), 2-methylpentan-3-amine (G15), methyl 2-isopropyl-3-methybut-2-enoate (G17) and *N*-enthyl-*N*-isopropylprop-1-en-2-amine (G18). Thus, these toxic fragments should be considered as structural alerts (SAs) for the ototoxicity, which may help medicinal chemists rationally design and select chemicals with the best prospects to be effective and safe.

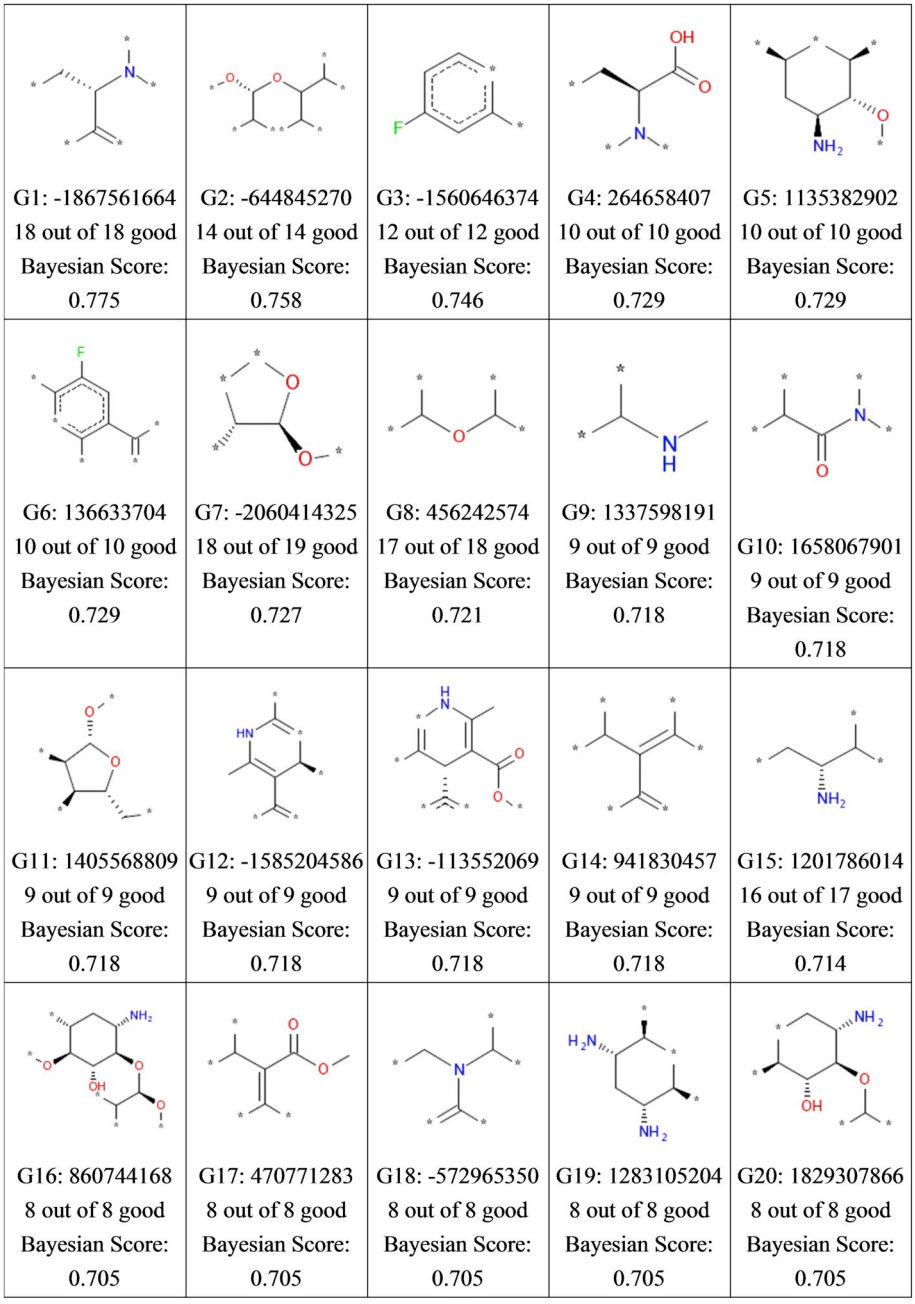
4.3. Comparison with other prediction models of chemical-induced ototoxicity

Computational prediction models for different types of toxicities assessment have been widely reported. However, for drug-induced ototoxicity, as far as we know, only Zhou et al. (2014) published support vector machine (SVM) prediction models for drug-induced ototoxicity with using 32 molecular descriptors, which produced 85.33% and 83.05% overall prediction accuracies for two independent test sets, respectively. In this investigation, the naïve Bayes prediction model (NB-1) was developed with using a larger dataset, which gave 90.2% overall prediction accuracy for the training set and 88.7% for the external test set. It was obvious that the prediction accuracies of the naïve Bayes classification model (NB-1) established in this investigation were higher than that of the reported SVM model. Furthermore, 7 molecular descriptors carrying information on chemicals with potential ototoxicity and some structural alerts (SAs) for the ototoxicity were identified, which would provide guidance for medicinal chemists working in lead optimization.

Moreover, in order to directly compare the prediction performance of the NB-1, the RP prediction model was constructed, in which the same datasets and descriptors applied in the development of NB-1 were employed. The minimum number of samples at each node was set to 8, and the maximum tree depth was designed as 8. The detailed prediction results of the RP model were displayed in Table 4. For the training set, the RP model gave 49.2% sensitivity (SE), 91.6% specificity (SP), 76.9% positive predictive value (PPV), 76.1% negative predictive value (NPV), 70.4% balance accuracy (BA) and 76.3% overall prediction accuracy (*Q*). The MCC value of the training set was 0.465. For the test set, the RP model produced 64.4% sensitivity (SE), 88.1% specificity (SP), 64.9% positive predictive value (PPV), 87.8% negative predictive value (NPV), 76.2% balance accuracy (BA) and 82.0% overall prediction accuracy (*Q*). The MCC value for the test set was 0.526. From the prediction results of the training set and test set, it was obviously observed that the predictive performance of the RP model was significantly lower than that of the NB-1 model. Especially, the RP model cannot correctly identify the ototoxic agents in the training set (SE, 49.2%) and test set (SE, 64.4%).

4.4. Analysis of the misclassified compounds by naïve Bayes model (NB-1)

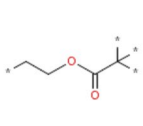
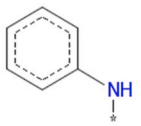
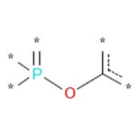
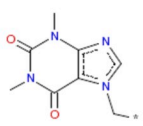
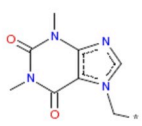
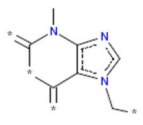
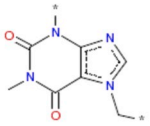
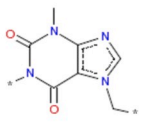
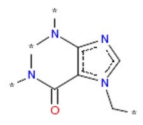
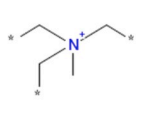
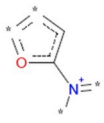
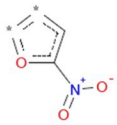
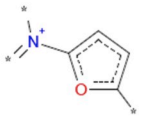
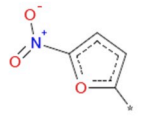
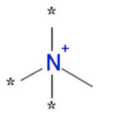
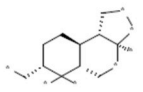
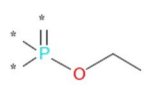
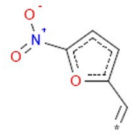
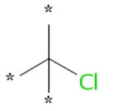
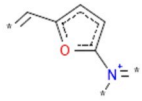
Although the established NB-1 model could successfully discriminate between ototoxicants and non-toxicants, 51 agents in the external test set were still wrongly predicted. The structures of these misclassified compounds in the external test set were listed in Supplementary material (Fig. S1 and S2). Further analysis of the structure characteristics of these misclassified compounds in the external test set, among the possible reasons there are 1) the intrinsic limitation of the naïve Bayes classifier based on the fingerprints may lead to these misclassifications. As can be seen from Fig. S1 and S2, some non-ototoxic features appeared in some misclassified toxicants, and some ototoxic features occurred in some misclassified non-toxicants, which may tend to be wrongly classified. 2) The structural information derived from ECFP_14 may not cover all dataset. As shown in Fig. S1 and S2, the ECFP_14 produced fragments were not presented in some misclassified agents. For example, the xylometazoline was shown to be non-ototoxic in a chinchilla animal model (Daniel et al., 2012), but which was misclassified as ototoxicant. 3) Some misclassified ototoxicants and non-ototoxicants may be explained by the presence of a few activity cliffs ('high' structure similarity and 'high' activity difference), which may distort the prediction models. As shown in Table 6, the fragment quinoline presented in misclassified No. 1, No. 2 and No. 3 compounds. The No. 1 compound belongs to ototoxic class, but No. 2 and No. 3 compounds belong to non-ototoxic class. Similarly, the dodecahydro-1H-cyclopenta[*a*]naphthalene fragment appeared in the misclassified non-ototoxic compounds (No. 4, No. 5, No. 6 and No. 7) and ototoxic agent (No. 8). In addition, the No. 9, No. 10 and No. 11compounds have similar structures, however, the non-ototoxic No. 10 and No. 11compounds were wrongly identified, but the No. 9 agent was correctly predicted. Furthermore, both the non-toxic No. 12 compound and ototoxic No. 13 compound contained the 5*H*-dibenzo [*a*, *d*] annulene fragment, but the No. 13 compound was misclassified, and the No. 12



(a)

Fig. 3. Some molecular fragments that important for ototoxicity produced by the ECFP_14 fingerprint descriptors. (a) The top 20 ototoxic substructures generated as good features from ECFP_14. Each panel shows the naming convention for each fragment, the numbers of molecules it is present in that are ototoxicants, and the Bayesian score for the fragment. (b) The top 20 non-toxic substructures produced as bad features from ECFP_14. Each panel shows the naming convention for each fragment, the numbers of molecules it is present in that are ototoxicants, and the Bayesian score for the fragment.

was correctly identified. In addition, the 9*H*-purine group presented in No. 14 and No. 15 compounds, but they belonged to different classes. The No. 14 agent was correctly predicted ototoxicant, but non-toxic No. 15 compound was misclassified. The phenomena of the similar compounds with very different biological activity have been observed in many previous studies (Dimova and Bajorath, 2016; Bajorath, 2014; Medina-Franco, 2013; Lei et al., 2017). The possible reason is that some specific fragments and their steric positions in the compounds may significantly influence the ototoxicity of the compounds. In this research, the molecular fingerprints produced by ECFP-14 could characterize the important structural fragments favorable or unfavorable for ototoxicants, but they don't fully characterize the molecular connectivity and its related features. Thus, given the importance of the implications of activity cliffs, molecular fragment connectivity descriptors representing group connectivity and adjacency should be considered for QSAR modeling, which may minimize the 'apparent' or 'false' activity cliffs. 4) The selected 7 molecular features were not able to describe the property of agents related to ototoxicity. For example, the descriptors of O_count, Num_H_Donors, Num_Rings 6, Num_Aromatic Rings and SC_0 only describe the number existing in compounds, but they cannot point out their

				
B1: 1135336976 0 out of 14 good Bayesian Score: -1.950	B2: 547788473 0 out of 13 good Bayesian Score: -1.887	B3: 1310520534 0 out of 11 good Bayesian Score: -1.747	B4: -1796400495 0 out of 11 good Bayesian Score: -1.747	B5: -697484771 0 out of 11 good Bayesian Score: -1.747
				
B6: -1577450369 0 out of 11 good Bayesian Score: -1.747	B7: 296204749 0 out of 11 good Bayesian Score: -1.747	B8: -805053206 0 out of 11 good Bayesian Score: -1.747	B9: -976437083 0 out of 11 good Bayesian Score: -1.747	B10: 1897502700 0 out of 10 good Bayesian Score: -1.669
				
B11: -1956181605 1 out of 22 good Bayesian Score: -1.656	B12: -1740976595 1 out of 22 good Bayesian Score: -1.656	B13: -1769453800 1 out of 22 good Bayesian Score: -1.656	B14: 790643813 1 out of 22 good Bayesian Score: -1.656	B15: 862647217 2 out of 32 good Bayesian Score: -1.595
				
B16: 170867520 0 out of 9 good Bayesian Score: -1.584	B17: 1890678861 0 out of 9 good Bayesian Score: -1.584	B18: 52266124 1 out of 19 good Bayesian Score: -1.524	B19: 106008533 1 out of 19 good Bayesian Score: -1.524	B20: -43486583 1 out of 19 good Bayesian Score: -1.524

(b)

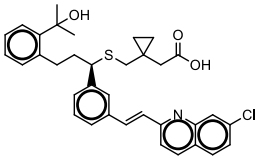
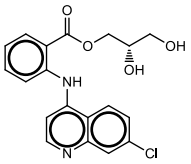
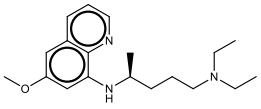
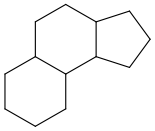
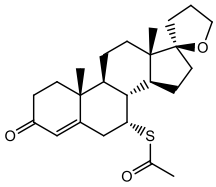
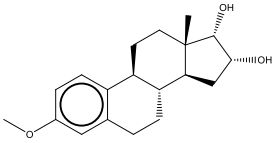
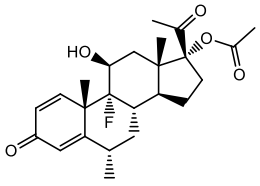
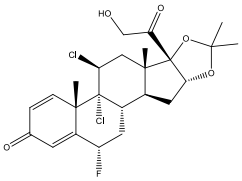
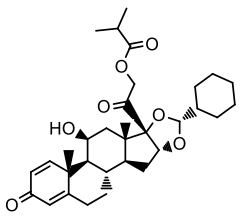
Fig. 3. (*continued*)

steric positions in the compounds. For these similar compounds with different biological activity, more precise descriptors representing feature steric positions should be considered in QSAR model. 5) The ototoxic agents were collected from a variety of sources, which may lead to some ototoxic data uncertainty. Thus, more accurate data should be provided in the future, which may improve the performance of the established prediction models. 6) At last, other non-naïve classifiers should be considered in the future, that is one in which the importance for classification of a given descriptor variable depends on the value taken by another descriptor variable.

5. Conclusions

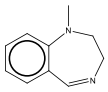
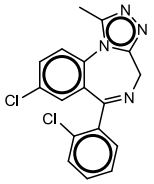
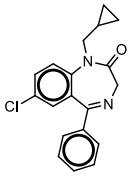
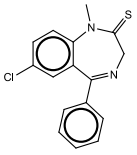
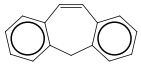
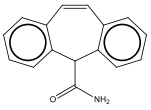
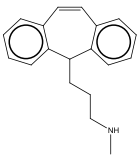
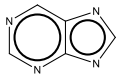
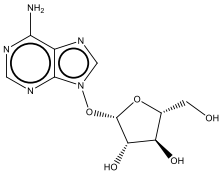
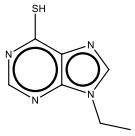
In this research, the *in silico* prediction model of ototoxicity was developed based on 2612 diverse chemicals by using naïve Bayes classifier approach. The genetic algorithm method was employed to find the important molecular descriptors related to ototoxicity, and the ECFP_14 fingerprint descriptors were applied to generate the ototoxic/non-toxic fragments. The established naïve Bayes prediction model achieved overall prediction accuracy of 90.2% for the training set and 88.7% for the external test set. In addition, a set of 7 molecular descriptors considered as important for ototoxicity and some structural alerts for ototoxicity were identified. We hope the established naïve Bayes prediction model should be employed as promising tool for screening the drug-induced ototoxicity in drug development, and these obtained important information of ototoxic chemical structures could provide theoretical guidance for hit and lead optimization in drug design.

Table 6
Examples of activity cliffs in the test set.

Fragments	Molecules	No	Name	Ototoxicity	Prediction
		1	Montelukast	YES	FN
		2	Glafenine	NO	FP
		3	Pamaquine	NO	FP
		4	Spiroxasone	NO	FP
		5	Epimestrol	NO	FP
		6	Fluorometholone acetate	NO	FP
		7	Flucloronide	NO	FP
		8	Ciclesonide	YES	FN

(*continued on next page*)

Table 6 (*continued*)

		9	Triazolam	YES	TP
		10	Prazepam	NO	FP
		11	Sulazepam	NO	FP
		12	Citenamide	NO	TN
		13	Protriptyline	YES	FN
		14	Vidarabine	YES	TP
		15	9-ethyl-6-mercaptopurine	NO	FP

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tiv.2020.104812.

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