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Original Article

Classification of 5-HT_{1A} receptor agonists and antagonists using GA-SVM method

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Aim: To construct a reliable computational model for the classification of agonists and antagonists of 5-HT_{1A} receptor.
Methods: Support vector machine (SVM), a well-known machine learning method, was employed to build a prediction model, and genetic algorithm (GA) was used to select the most relevant descriptors and to optimize two important parameters, C and r of the SVM model. The overall dataset used in this study comprised 284 ligands of the 5-HT_{1A} receptor with diverse structures reported in the literatures.
Results: A SVM model was successfully developed that could be used to predict the probability of a ligand being an agonist or antagonist of the 5-HT_{1A} receptor. The predictive accuracy for training and test sets was 0.942 and 0.865, respectively. For compounds with probability estimate higher than 0.7, the predictive accuracy of the model for training and test sets was 0.954 and 0.927, respectively. To further validate our model, the receiver operating characteristic (ROC) curve was plotted, and the Area-Under-the-ROC-Curve (AUC) value was calculated to be 0.883 for training set and 0.906 for test set.
Conclusion: A reliable SVM model was successfully developed that could effectively distinguish agonists and antagonists among the ligands of the 5-HT_{1A} receptor. To our knowledge, this is the first effort for the classification of 5-HT_{1A} receptor agonists and antagonists based on a diverse dataset. This method may be used to classify the ligands of other members of the GPCR family.

Keywords: 5-HT_{1A} receptor; support vector machine; genetic algorithm; agonist; antagonists

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Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) mediates many of physiological responses and pathological processes in both the peripheral and central nervous system and has diverse effects on appetite, sleep and general metabolism[1]. The dysfunction of serotoninergic neurotransmission causes many psychiatric disorders such as depression, anxiety and migraine[2]. Serotonin exerts its functions primarily by interacting with different types of serotonin receptors (5-HT receptors)[3–6]. The 5-HT receptors are a subfamily of G protein-coupled receptors (GPCRs) with the exception of the 5-HT_3 subtype, which is a ligand-gated ion channel[7]. At least 14 distinct 5-HT receptors have been identified to date that can be divided into seven subtypes (5-HT_1~5-HT_7) based on the molecular cloning, amino acid sequence, pharmacological properties and signal transduction[8]. The 5-HT_{1A} receptor, which is mainly distributed in the frontal cortex, septum, amygdala, hippocampus, and hypothalamus[9, 10], is one of the best characterized members in the family and is a crucial modulator of serotonergic signaling in the central nervous system[11].

Accumulating results indicate that the 5-HT_{1A} receptor participates in the regulation of various physiological and pathophysiological processes such as psychosis, cognition, feeding/satiety, temperature regulation, depression, anxiety, sleep, pain perception and sexual activity[12, 13]. It has become one of the most attractive targets for the development of drugs treating numerous neurological and psychiatric disorders. Currently, five drugs primarily targeting this receptor have already been launched, and dozens of others are in various clinical stages. Among these launched drugs, buspirone is the earliest 5-HT_{1A} receptor agonist that was launched in 1985 by Bristol-Myers Squibb (BMS) for the management of anxiety disorders. Although no 5-HT_{1A} antagonists have been launched, previous studies have shown that 5-HT_{1A} receptor antagonists may be useful in the treatment of Alzheimer's disease and other cognition disorders[14]. In view of the significant differences in physiological functions between the 5-HT_{1A} receptor agonists and antagonists, identification of agonistic or antagonistic properties of 5-HT_{1A} receptor ligands has become

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an important issue for drug development.

Some experimental methods have been established to identify the function of known ligands[15, 16]. However, these methods are time-consuming or expensive. Furthermore, the assays cannot be employed unless a compound is available. Therefore, a reliable computational model would be beneficial to accurately predict the physiological function of a compound before it is synthesized. To the best of our knowledge, no such model has been reported to date.

In this study, a genetic algorithm optimized the support vector machine (GA-SVM) method was adopted to construct a computational model for the identification of agonists or antagonists of the 5-HT_{1A} receptor using 259 agonists and antagonists collected from the literatures. The constructed SVM model displayed high predictive accuracy for training and test sets. The application of the model to an external dataset that comprised 25 recently reported ligands revealed that our predicted data were in good agreement with their biological functions of the reported ligands, demonstrating that our model was reliable for identifying agonists and antagonists of the 5-HT_{1A} receptor. This approach may also be employed to construct models to predict agonists or antagonists of other GPCR members.

Materials and methods

Data sets

A total of 259 5-HT_{1A} receptor ligands were collected from previous studies[1, 15–47], which are composed of 137 agonists and 122 antagonists with diverse structural classes such as aminotetralins, indolylalkylamines, ergolines, aporphines, arylpiperazines and aryloxyalkylamines (Tables S1 and S2 in *Supplementary Information*). Because we aimed to build a binary classifier, the partial agonists of the 5-HT_{1A} receptor were classified as agonists. When provided with biological data containing conflicting information for the same compound from different research groups, we used the latest results or the results from the research group with a long history of studying 5-HT_{1A} receptor ligands as our raw data. All of the 259 function-known ligands were randomly divided into training and test sets with the ratio of 4:1 (207:52) (Table 1). The training set was used to develop the prediction model, whereas the test set was used to assess the performance of the generated model. The structures of these compounds were created and optimized using Sybyl6.8[48].

Table 1. Number of agonists and antagonists in training set and test set.

Data sets	Number of agonists	Number of antagonists	Total number
Training set	109	98	207
Test set	28	24	52

Support vector machine

The support vector machine (SVM), which was originally developed by Vladimir Vapnik *et al*, is based on the structural risk minimization principle from the statistical learning theory and is a supervised learning method that can be applied to classification and regression[49]. Simply speaking, a SVM model is constructed based on a given set of training inputs belonging to two different classes. Then, the model is used to predict the class of a new input. A data input is regarded as a multi-dimensional vector, and the goal is to determine a hyperplane to separate the inputs, which are the sets of agonists or antagonists in this study. In particular, the popular Library for Support Vector Machines (LIBSVM2.89) was employed in this study[50].

There are two parameters, C and r, that must be carefully adjusted to develop a robust SVM model. C is a global parameter, which regulates the trade-off between maximization of the margin and minimization of the training error. Small C values are prone to highlight the margin and overlook the outliers in the training set. However, large C values may lead to overfitting of the training set. The parameter r indicates the radial basis function (RBF), which is the kernel function used in this study[51]. Here, we optimized the value of C and r using our in-house method (detailed below) to build the best classification model.

Molecular descriptors

Molecular descriptors are generally used for quantitative representation of the structural and physicochemical features of compounds[52, 53]. Depending on the 3D structure of each compound, 292 molecular descriptors including topological, graph-theoretical, quantum-chemical and electro-topological state (E-state) descriptors were calculated using Discovery Studio 2.1[54]. In addition, the value of every descriptor was scaled to [-1, 1] (see *Supplementary Information,* Excel S1).

Feature selection and parameter optimization

Usually, only a few of the calculated molecular descriptors are essential to develop a SVM model. To select the most important descriptors and optimize model parameters simultaneously, an in-house program was coded in our laboratory using genetic algorithm (GA)[55]. GA is a method that randomly initializes a population of solutions and then improves it through repetitive operations of mutation, crossover and selection. Each possible solution is referred to as a chromosome, which consists of two parts, the feature mask and the SVM parameters (C and r). The value of the feature mask is 0 or 1, where 0 represents the corresponding descriptor is abandoned while 1 indicates to keep the descriptor. Although the value of C and r are real numbers, only specific discrete values were considered in this study, where C and r were represented as 2^m and 2^n with m and n integers.

When developing the classification model, 5-fold cross-validation was adopted to explore the reliability of the statistical models. The training set of 207 ligands in this study was randomly split into five subsets of approximately equal size. In each validation, one subset was used for test while the rest four were used for training the model. This process was

repeated five times so that each subset could be used for the prediction once.

Model validation

After the model was built, we adopted different means to evaluate its performance. Receiver-operating-characteristics (ROC) curve is generally used to assess the classification power of computational models[56, 57]. To plot a ROC curve, only the true positive rate (TPR) and false positive rate (FPR) were required. TPR, which is also called sensitivity and is calculated with equation (1), defines how many true positive results appear among all of the positive inputs during prediction. Instead, FPR determines how many false positive results emerge among all of the negative inputs. FPR is equal to (1–specificity), where the specificity is calculated with equation (2).

$$Sensitivity = \frac{TP}{TP+FN} \quad (1)$$

$$Specificity = \frac{TN}{TN+FP} \quad (2)$$

In these equations, TP represents the number of correctly predicted agonists, TN represents the number of correctly predicted antagonists, FP represents the number of antagonists that are incorrectly predicted as agonists and FN represents the number of agonists that are incorrectly predicted as antagonists.

The quality of our SVM model was also measured using the Matthews correlation coefficient (MCC), which is defined by equation (3)[58, 59]. It returns a value between –1 (worst model) and 1 (perfect model) while 0 represents a random model.

$$MMC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP+FP)(TP+FN)(TN+FP)(TN+FN)}} \quad (3)$$

To fully examine the performance of the developed model, the overall accuracy is calculated using equation (4).

$$Accuracy = \frac{TP+TN}{TP+FP+TN+FN} \quad (4)$$

Results

Feature selection and model performance

Using our in-house feature selection program, 13 descriptors were finally selected (Table 2), which was roughly divided into five classes. The optimized values of *C* and *r* were both 1 in the SVM model with a cross-validation r^2 of 0.826. As shown in Table 3 (before refinement), the overall predictive accuracy, sensitivity and specificity for training and test sets were all higher than 0.8, indicating that the developed model was reliable and robust. Indeed, the calculated MCC was 0.783 for the model.

To view the results more intuitively, the quality of the results was illustrated using ROC plots (Figure 1). The (0,1) point in the upper left corner of the ROC space represented 100% sensitivity (no false negatives) and 100% specificity (no false positives). A random classifier would give us a diagonal line (the so-called line of no-discrimination) from the left bottom to the top right corner. Finally, we explored another parameter that was represented as the Area-Under-the-ROC-Curve (AUC). The AUC values for the training and test sets were 0.883 and 0.906, respectively.

Model refinement

To further improve the performance of the developed model, the probability estimate factor was used as a criterion to remove those ambiguous compounds with a threshold of the factor less than 0.7. Thus, two probability estimates, namely agonist probability estimate and antagonist probability estimate, were calculated for each compound, and the sum of them was equal to 1. The larger probability estimate

Table 2. List of optimized 13 molecular descriptors used in the SVM and their descriptions and classes.

Descriptor	Description	Descriptor Class
HOMO_Eigenvalue_VAMP	The eigenvalue of the highest occupied molecular orbital	A
QsumHal_Propgen_VAMP	The sum of the electrostatic potential-derived atomic charges on halogen atoms	A
No_of_surface_points_with _+ve_ESP_Propgen_VAMP	The number of surface points with positive electrostatic potential	A
Octupole_XXY_VAMP	The XXY component of octupole moment	A
ES_Sum_sssCH	The sum of the electrotopological state value for atom type sssCH	B
ES_Sum_aaaC	The sum of the electrotopological state value for atom type aaaC	B
ES_Sum_dsN	The sum of the electrotopological state value for atom type dsN	B
ES_Sum_aaN	The sum of the electrotopological state value for atom type aaN	B
ES_Sum_sssN	The sum of the electrotopological state value for atom type sssN	B
Num_Aromatic Bonds	Bonds in aromatic ring systems	C
Energy	The energy of the molecule's current 3D conformation	D
Shadow_XZ	The area of the molecular shadow in the xz plane	E
Shadow_YZ	The area of the molecular shadow in the yz plane	E

A, semi-empirical quantum mechanical properties (VAMP optimized with AM1, propgen method); B, estate keys (s, sing bond; d, double bond; t, triple bond; a, aromatic bond); C, molecular property counts; D, molecular properties; E, shadow indices.

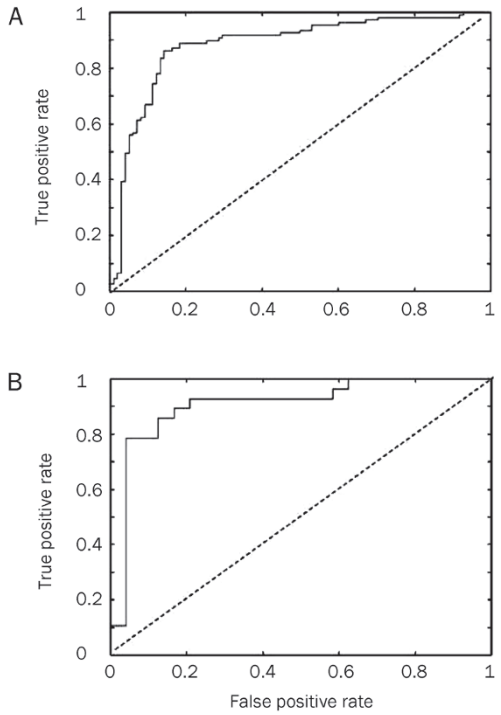

Figure 1. ROC curves for training set (A) and test set (B). The solid line represents ROC curve and the area under the curve is characterized by AUC, whose value for training set and test set are 0.883 and 0.906, respectively. The dash line is diagonal line, which describes a random model.

is regarded as the probability estimate factor of a specific compound. Figure 2 shows the probability estimate distribution for compounds in the training and test sets. There were 195 compounds in the training set and 41 compounds in the test set that remained in the refined datasets, demonstrating that the majority of the compounds had a probability estimate higher than 0.7. Then we reevaluated our model using the refined datasets, which yielded an improved accuracy, sensitivity and specificity (>0.9, after refinement in Table 3).

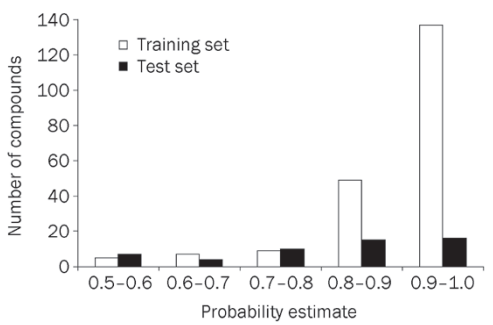

Figure 2. Probability estimate for training set (white) and test set (black). There are only 12 ligands with probability estimate lower than 0.7 among all 207 ligands in training set, so the threshold of probability estimate factor is chosen to be 0.7. Moreover, the probability estimate of 11 ligands from test set are less than this threshold, indicating that the predicted result may be unreliable.

Table 3. The value of accuracy, sensitivity and specificity before and after refinement.

Parameters	Before refinement		After refinement	
	training set	test set	training set	test set
Accuracy	0.942	0.865	0.954	0.927
Sensitivity	0.936	0.893	0.943	0.917
Specificity	0.949	0.833	0.967	0.941

Application of the SVM model to an external dataset

To validate the reliability of our SVM model for identifying agonists and antagonists of the 5-HT_{1A} receptor, we applied the model to an external dataset including 25 ligands that were collected from very recently published literatures. In conclusion, 15 compounds were predicted to be antagonists, and 3 compounds were predicted as agonists with probability estimate higher than 0.7 (Table 4).

Discussion

As shown in Table 2, 13 molecular descriptors were selected as the most relevant descriptors for discriminating between 5-HT_{1A} receptor agonists and antagonists, including VAMP/AM1 semi-empirical quantum-chemical, electro-topological state (E-state), molecular property and shadow index descriptors. Several selected descriptors reflected the corresponding structural information that was closely related to the function of these ligands. For example, the E-state indices were efficient descriptors to describe the affinity of 5-HT_{1A} receptor antagonists[60]. Our results demonstrate that another important descriptor, the "number of surface points with positive electrostatic potential", is in agreement with the data that most of the agonists or antagonists of 5-HT_{1A} receptor are positively charged. None of the descriptors alone could completely describe the differences between agonists and antagonists. However, the collective use of the descriptors yielded a more accurate model. Thus, a group of diverse, comprehensive and representative descriptors were used to develop a powerful SVM model which could effectively distinguish agonists from antagonists of 5-HT_{1A} receptor.

Another highlight of our study was the consideration of the probability estimate factor while establishing the SVM model. For example, an agonist with the probability estimate of 0.9 is more likely to be an agonist than the one with the probability of 0.6, which is also true for antagonists. Based on the probability estimate distribution for the compounds of the training set, the threshold of the probability estimate was set to 0.7 for more reliable classification. Then, the compounds with probability estimate less than 0.7 were removed from training and test sets. The predictive accuracy for the refined datasets was significantly increased, especially for the test set, from 0.865 to 0.927. These results demonstrate an improved predictive power after introducing the probability estimate factor. Moreover, differences in accuracy, sensitivity, and specific-

Table 4. Detailed predicted results of 25 external compounds.

Compound name	Predicted result	Probability estimate	K_i (nM)[d]	Name in reference
HT01	antagonist	0.839	1.69	1[a]
HT02	antagonist	0.918	5.19	2[a]
HT03	antagonist	0.868	4.59	3[a]
HT04	antagonist	0.789	4.64	4[a]
HT05	antagonist	0.849	2.75	5[a]
HT06	antagonist	0.870	4.1	6[a]
HT07	antagonist	0.833	11.1	7[a]
HT08	antagonist	0.678	8.53	8[a]
HT09	antagonist	0.806	6.15	9[a]
HT10	antagonist	0.790	2.87	10[a]
HT11	antagonist	0.868	480	5[b]
HT12	agonist	0.844	120	8[b]
HT13	agonist	0.843	60	10[b]
HT14	antagonist	0.846	890	16[b]
HT15	antagonist	0.781	7980	19[b]
HT16	antagonist	0.872	2780	21[b]
HT17	agonist	0.848	4210	23[b]
HT18	agonist	0.532	36.1	17[c]
HT19	antagonist	0.660	NA[e]	23[c]
HT20	antagonist	0.666	NA[e]	27[c]
HT21	antagonist	0.704	41.5	29[c]
HT22	antagonist	0.647	NA[e]	33[c]
HT23	antagonist	0.585	12.7	38[c]
HT24	antagonist	0.708	11	40[c]
HT25	antagonist	0.612	5.14	42[c]

[a] Compounds collected from reference 61.
[b] Compounds collected from reference 62.
[c] Compounds collected from reference 63.
[d] Data retrieved from the corresponding reference.
[e] No activity data was detected.

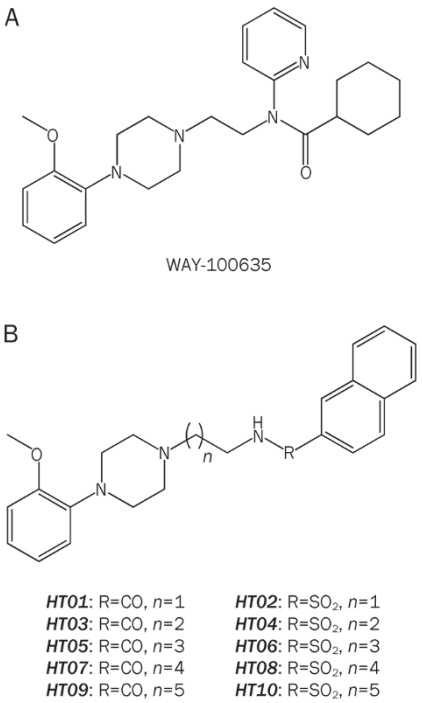

Figure 3. Chemical structures of WAY-100635 (A) and compounds ***HT01~HT10*** (B). As shown, they are structurally similar, all of them containing a [4-(2-methoxyphenyl)-1-piperazinyl]ethyl structure fragment. WAY-100635 is believed to act as a selective 5-HT_{1A} receptor antagonist, so compounds ***HT01~HT10*** may also be antagonists of 5-HT_{1A} receptor.

ity between the training and test sets after refinement were much smaller than those before refinement, suggesting that a more balanced model between the training and test sets was achieved.

The probability estimate is an important parameter for judging the reliability of the predicted result. For instance, ***HT01~HT10***, a series of carboxamide and sulfonamide alkyl[61], were predicted to be antagonists with high probability. Indeed, they are structurally similar to WAY-100635 (Figure 3), a well-known antagonist of the 5-HT_{1A} receptor, indicating that ***HT01~HT10*** are likely to function as antagonists of the 5-HT_{1A} receptor. These results show that our SVM model has an instructive role for exploring agonists and antagonists of the 5-HT_{1A} receptor. Besides, a group of newly discovered N-phenylpiperazine derivatives, ***HT11~HT17***, were predicted to function as agonists or antagonists with high probability estimate. For example, compound ***HT13*** was predicted to be an agonist by our model, which was also believed to stimulate 5-HT_{1A} receptor activity like an agonist by other research group[62], indicating that similar structures may play different roles in regulating the function of the receptor.

On the other hand, those compounds with only moderate or poor 5-HT_{1A} receptor affinity in biological tests were predicted to be binders with lower probability estimates. For instance, the probability estimates of compounds ***HT18~HT22***, which are weaker binders[63], were approximately 0.7. Therefore, further biological research on these compounds may not be urgent. Similarly, the predicted results for compounds ***HT23~HT25*** with lower probability estimates are contrary to the known biological functions. Actually, they had been proved to be weak agonists or partial agonists of the 5-HT_{1A} receptor[63]. These conflicts indicate that our SVM model may have problems with applicability of chemical space similar to other computational models and requires further optimization.

In summary, based on 13 molecular descriptors that were derived from previously known agonists and antagonists, we developed a robust SVM model with great predictive capability. Moreover, the predictive accuracy for the training and test sets (especially for the test set) were significantly increased when we considered the compounds with probability estimate higher than 0.7. Then we applied the model to an external dataset for validation, which confirmed that our GA-SVM method is effective for the classification of agonists and antagonists of the 5-HT_{1A} receptor. The strategy and methods used in this study may be extended to other GPCR members.

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Author contribution

Prof Wei-liang ZHU designed and supervised the research and revised the manuscript. Xue-lian ZHU performed the research, analyzed data and wrote the manuscript. Prof Heyao WANG and Zhi-jian XU helped with parts of the research design. Hai-yan CAI, Yong WANG, and Prof Ao ZHANG helped to perform the research and revise the manuscript.

Supplementary information

Table S1 and Table S2 show the chemical structures of 137 agonists and 122 antagonists, respectively. The chemical structures of 25 ligands are displayed in Table S3. All of the values of the molecular descriptors are shown in Excel S1. There are 6 sheets in Excel S1 containing 3 sheets of raw data and 3 sheets of scaled data. Supplementary information is available at the Acta Pharmacologica Sinica's website.

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