A QSAR Study for Modeling of 8-Azaadenine Analogues Proposed as A1 Adenosine Receptor Antagonists Using Genetic Algorithm Coupling Adaptive Neuro-Fuzzy Inference System (ANFIS)

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A quantitative structure activity relationship (QSAR) study of 8-azaadenine, as antagonists for the A1 receptor, is described. A genetic algorithm (GA) method was used as the feature selection tool, and an adaptive neuro-fuzzy inference system (ANFIS) was employed for feature mapping. The best descriptors (GATS4v and BELv7) were applied to train the ANFIS model. The optimum number and shape of related functions were obtained through a subtractive clustering algorithm. The ability and robustness of the GA-ANFIS model in predicting the affinity of 8-azaadenine derivatives (pK_i) are illustrated by validation techniques of Leave One Out, heuristic and randomized methods. The results have indicated that the proposed model of ANFIS in this work is superior over two other methods, radial basis function (RBF) and multiple linear regression (MLR).

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Introduction

Quantitative structure-activity/property relationship (QSAR/QSPR) methods are common and rather successful techniques in drug design. However, in cases of complex relationships, conventional QSAR/QSPR methods often lead to insufficient or misleading information because of nonlinear relationships within the data set.¹ One possibility of overcoming the difficulties of such nonlinearities in QSAR/QSPR studies is the use of artificial neural networks (ANN), which has gained interest in the field of drug design.²⁻⁸ On the other hand, Fuzzy logic is a problem-solving technique that defines and generates responses based on ambiguous, incomplete and imprecise information, and fuzzy systems have fascinated the growing attention and interest in bioinformatics applications, decision-making studies, pattern recognition and data analysis.^{9,10} The adaptive neuro-fuzzy inference system (ANFIS) is the achievement of a fuzzy inference system in the form of a multi-layer neural network.^{11,12} ANFIS can construct an input-output mapping based on a given initial fuzzy system and available input-output data pairs by using a learning procedure. The adaptive network simulates a fuzzy inference system represented by fuzzy if-then rules. The hybrid network of the ANFIS system is functionally equivalent to Sugeno's inference mechanism.¹³ Recently, some applications of ANFIS in QSAR modeling have been reported.¹⁴⁻¹⁷

A genetic algorithm is an optimization method that can be used to solve different chemical problems.¹⁸ This method searches the solution space of a function through the use of simulated evolution, *i.e.* the survival of the fittest strategy. The

genetic algorithm has been shown to solve optimization problems and exponentially exploring promising areas through mutation, crossover and selection operators applied to individuals in the population. Roger and Hopfinger¹⁹ and Luke²⁰ combined genetic algorithms with regression analysis. Sutter *et al.*²¹ developed a generalized simulated annealing method that makes use of a simulated annealing and a neural network. Herro and Ortiz²² reported the application of genetic algorithm (GA)-based variable selection in polarography and stripping voltammetry. Also, there are many published reports about the application of GA in multi-component analysis by spectroscopy.^{23,24}

Adenosine receptors (ARs) are members of the P1 family, and are coupled with more subtypes of G proteins, which regulate the activity of adenylyl cyclase and other effector systems, including calcium or potassium ion channels, phospholipase A or C, and guanylate cyclase.²⁵ They are classified as A1, A2a, A2b, A3 subtypes, and have been characterized using selective ligands. The different receptor subtypes can be distinguished on the basis of their agonist and antagonist specificities. The subtypes differ in their influences on cell metabolism and their tissue distribution.²⁶ A1 receptors are widely distributed in the central nervous system and in many peripheral tissues, and mediate diverse physiological effects. A1 antagonists are studied as antihypertensives and potassium sparing diuretics with kidney protection properties,²⁷ and could be useful in Alzheimer's disease and other CNS disorders,²⁸ and in cardiac therapy and kidney diseases.²⁹ Great numbers of A1 adenosine receptor ligands have been synthesized, in which the substituent groups at the 3, 6 and 9 positions of the 8-azaadenine nucleus were changed. Some of these compounds were shown to be very effective, because their affinity (K_i values) was found in the range of nanomolar units.³⁰⁻³⁸ A number of models of QSAR have been developed about the QSAR modeling of A adenosine receptors.^{39,40}

In this paper, we describe a QSAR study based on ANFIS

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Fig. 1 ANFIS architecture.

presented for the prediction of some 8-azaadenine analogues proposed as A1 adenosine receptor antagonists synthesized by Giorgi *et al.*³⁸ It has been demonstrated that ANFIS is a useful tool for QSAR modeling, comparable to the multiple linear regression (MLR) and radial basis function (RBF) methods. Finally, the accuracy of the proposed model was illustrated using Leave One Out, heuristic and randomized techniques.

Theory

ANFIS

Among various fuzzy inference systems, Takagi-Sugeno (TS) systems⁴¹ have been successfully applied for datadriven-based fuzzy modeling. The TS model consists of a set of local input-output relations that describe the overall system. The rules in a first-order TS model have the following structure:

R_i : (if x_1 is A_{i1} , ..., and x_n is A_{in}) then

$$Y_i = a_i^T x + b_i \quad (i = 1, 2, 3 \dots k)$$

Hence R_i is the i th rule in the rule base, $x = [x_1, x_2, \dots, x_n]^T$ is the input (antecedent) vector, and $A_{i1}, A_{i2}, \dots, A_{in}$ are the fuzzy sets defined for the respective antecedent variables. The rule consequent Y_i is an affine combination of the inputs with parameters a_i and b_i .⁴²

An ANFIS system can be considered to be an implementation of a TS system in neural-network architecture. In the following, we briefly sketch an outline of the ANFIS system by using a model with two inputs as an example⁴³ (see Fig. 1). Let the inputs of the fuzzy system be x and y , and let the output be z . We consider a TS system with first-order consequents⁴⁴ and two rules, as follows:

Rule 1: If x is A_1 and y is B_1 , then $f_1 = p_1x + q_1y + r_1$.

Rule 2: If x is A_2 and y is B_2 , then $f_2 = p_2x + q_2y + r_2$.

To construct the adaptive system, five layers are used, as shown in Fig. 1. Each layer involves several nodes described by a node function. The circles in the network represent nodes that possess no variable parameters, while the squares represent nodes that possess adaptive parameters to be determined by network during training. The node function in each layer is described below.

Layer 1: The nodes in this layer represent the fuzzy sets in the antecedents of the fuzzy rules. It has parameters that control the shape and the location of the center of each fuzzy set. In this study, we choose $\mu_{A_i}(x)$ to be Gaussian with the height equal to 1. The membership function is given by

$$\mu_{A_i}(x) = e^{-(x-c_i)^2/2\sigma_i^2} \quad (1)$$

where c_i represents the center of the Gaussian function and σ_i represents the spread of the membership function. The outputs of this layer are the values of the antecedent membership functions corresponding to the fuzzified inputs of the system.

Layer 2: Every node in this layer computes the product of its inputs. The output of the layer is given by

$$w_i = \mu_{A_i}(x) \times \mu_{B_i}(y) \quad (i = 1, 2, \dots) \quad (2)$$

where μ_{A_i} and μ_{B_i} are the fuzzy sets defined for the variables x and y , respectively. The aim of this layer is to compute the degrees of activation (firing strength) of particular fuzzy rules.

Layer 3: The nodes in this layer normalize the firing strength of the rules by calculating the ratio of the i th rule's firing strength to the sum of all rules firing strengths by

$$w_i^* = \frac{w_i}{w_1 + w_2} \quad (i = 1, 2, \dots) \quad (3)$$

Layer 4: Nodes in this layer are adaptive, where each node function represents a first-order model with consequent parameters. Thus, the output from this layer is expressed by

$$O_i^4 = w_i^* f_i = w_i^* (q_i x + p_i y + r_i) \quad (4)$$

where w_i^* is the output of Layer 3, and $\{p_i, q_i, r_i\}$ is the parameter set.

Layer 5: This is the output layer where each node is fixed. The single node labeled Σ computes the overall output as the summation of all the inputs from the previous layer by

$$O_i^5 = \sum_i w_i^* f_i = \frac{\sum_i w_i f_i}{w_i} \quad (5)$$

Optimizing the values of the adaptive parameters is of vital importance for the performance of the adaptive system. In particular, the premise parameters in Layer 1 and the consequent parameters in Layer 4 need to be determined. Layer 1 parameters define the center and the spread of the antecedent membership function. Layer 4 parameters defined by the set $\{p_i, q_i, r_i\}$ corresponding to the consequent coefficients in Eq. (4). Jang¹² proposed a hybrid-learning algorithm for determining the parameters of an ANFIS model. A hybrid learning algorithm combines the gradient descent and least-square techniques for optimizing the network parameters. In this proposed approach, the output of the system, f , is written as

$$\begin{aligned} f &= \frac{w_1}{w_1 + w_2} f_1 + \frac{w_2}{w_1 + w_2} f_2 \\ &= w_1^* f_1 + w_2^* f_2 \\ &= (w_1^* x) p_1 + (w_2^* y) q_1 + (w_1^*) r_1 + (w_1^* x) p_2 + (w_2^* y) q_2 \\ &\quad + (w_2^*) r_2 \end{aligned} \quad (6)$$

where $(p_1, q_1, r_1, p_2, q_2, r_2)$ are the consequent parameters of the linear sub-systems. If the objective function to minimize is chosen to be minimization of the squared prediction errors, the objective function is linear in the consequent parameters. Therefore, the least-squares estimation can be used to determine these parameters, assuming that the Layer 1 parameters are fixed. Afterwards, the Layer 4 parameters can be fixed, and a back propagation approach is used to adapt the premise parameters in Layer 1. Iterating between optimizing the Layer 1

Table 1 Structures and experimental pK_i values of 8-azaadenine derivatives

R				
-CH ₃	A1 (0.4522) ^a	B1 (0.719)	C1 (1.6021)	D1 (1.8268)
-C ₂ H ₅	A2 (1.042)	B2 (0.3344)	C2 (1.9586)	
-nC ₃ H ₇	A3 (1.4318)	B3 (0.9957)	C3 (2.4048)	
-nC ₄ H ₉	A4 (1.9586)	B4 (1.1024)	C4 (2.3665)	
-C ₆ H ₁₃	A5 (1.8794)	B5 (1.5086)	C5 (1.6021)	D4 (2.1871)
	A6 (1.9586)	B6 (1.9586)	C6 (1.8239)	
	A7 (2.301)	B7 (1.6198)		D6 (2.1079)
	A8 (1.9586)	B8 (2.0458)	C8 (2.1739)	
	A9 (2.7959)	B9 (1.4685)	C9 (2.9393)	D8 (3.2218)
-CH ₂ CH ₂ OH	A10 (1.4202)	B10 (0.9355)	C10 (1.2676)	D9 (2.1675)
-CH ₂ CHOHCH ₃	A11 (2.1249)	B11 (1.4949)	C11 (2.3188)	
-CH ₂ CH ₂ CH ₂ OH	A12 (1.6696)	B12 (1.4815)	C12 (2.4318)	
	A13 (2.5686)	B13 (2.5086)	C13 (2.7328)	D12 (0.8327)
	A14 (1.9872)			

a. Numbers in parenthesis indicate the experimental pK_i values for the dataset molecules.

parameters and the Layer 4 parameters, the optimal values for all free parameters are computed.

Performance measurement

The major concerns of the validation are the evaluation, predictability and validity of the models, and the consistency of the results. One of the most common methods is to measure the root mean-square error (RMSE), which is the degree of distribution of the data. The RMSE can be calculated by the below-mentioned equation, where N denotes the number of data patterns, y_i^{pred} is the predicted output for pattern number i , and y_i^{obs} is the experimental output data as follows:

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^N (y_i^{\text{pred}} - y_i^{\text{obs}})^2} \quad (7)$$

The consistency of the ANFIS model was revealed by predictive Q^2 . The Q^2 values measure the goodness of the predictions of the held out cases exactly in the same way as R^2 does with the cases included in the modeling phase. However Q^2 is lower, and may be even negative if the predictions are worse than just using the average value of the response. The Q^2 value should be at least 0.3–0.4 in order to assess that the model has statistically significant prediction ability. In this study, the Q^2 values of the models were calculated by

$$Q^2 = 1 - \frac{\sum_{i=1}^N (Y_{\text{obs}} - Y_{\text{pred}})^2}{\sum_{i=1}^N (Y_{\text{obs}} - Y_{\text{mean}})^2} \quad (8)$$

where Y_{obs} and Y_{pred} are the observed and predicted affinity, respectively, and Y_{mean} is the observed average.

Experimental

Data set and descriptor generation

This study utilized a data set, comprised of 45 azaadenine derivatives, which their affinity toward the bovine A1 adenosine receptor (K_i values) was measured by a radioligand binding assay.³⁸ The affinity of an antagonist for its binding site (K_i), *i.e.* its ability to bind to a receptor, will determine the duration of the inhibition of agonist activity. The data set and corresponding observed values of pK_i of all molecules studied in this work are given in Table 1. The computations were carried out using programs written in MATLAB 6.5 by the authors. Prior to calculation of the molecular descriptors, the 3D structures of the studied compounds were optimized using semi-empirical quantum-chemical methods of AM1 implemented in the Gaussian 98 program package. Dragon 3.0⁴⁵ provides molecular descriptors that are divided into 18 logical blocks. We have calculated 16 molecular descriptor categories out of 18 included electronic, geometric and topological descriptors. The excluded two data sets (the functional group and atom-centered fragment descriptors) were eliminated because the program gave zero values for the compounds. Some of the descriptors generated for each compound encoded similar information about the molecule of interest. Therefore, it was desirable to test each descriptor and to eliminate those that show high correlation ($R > 0.95$) with each other. A total of 105 out of 1256 descriptors showed high correlation, and was removed from the next consideration. Subsequently, the method of stepwise partial least-squares (PLS) regression and genetic algorithm were used for selecting important descriptors.

The applied GA contained a population of 100 individuals,

Table 2 Names of descriptors chosen by GA and stepwise regression

No.	Input parameter
1	R3p (GETAWAY, R autocorrelation of lag3/weighted by atomic polarisibilities)
2	Dp (WHIM-D total accessibility index/weighted by atomic polarisibilities)
3	Mor25m (3-D MoRSE-signal 25/weighted by atomic mass)
4	GATS4v (2nd autocorrelations-Geary autocorrelation-lag4/weighted by atomic van der Waals volumes)
5	MATS7v (2nd autocorrelation-Moran autocorrelation-lag7/weighted by atomic van der Waals volumes)
6	BELv7 (BCUT-lowest eigenvalue n7 of burden matrix/weighted by atomic van der Waals volumes)

which evolved for 100 generations with PLS regression. Then by a comparison between the fitness values of individuals, the best model was chosen. Table 2 gives the names of all descriptors that were chosen by the GA technique and the stepwise regression method. The descriptors are derived from either two- or three-dimensional molecular structure indices based on BCUT and 2nd autocorrelation, 3D-MoRSE, WHIM, and GETAWAY descriptors. These classes of descriptors associate with the atomic masses, the van der Waals characteristics, the electrotopological states, and the Sanderson electronegativities of the 8-azaadenine derivatives. Constitutional descriptors are related to the number of atoms and bonds in each molecule. Topological descriptors include valence and non-valence molecular connectivity indices calculated from the hydrogen-suppressed formula of the molecule, encoding information about the size, composition, and the degree of branching of a molecule. Geometrical descriptors are calculated from 3D atomic coordinates of the molecule, and comprise the moments of inertia, shadow indices, molecular volumes, molecular surface areas, and gravitation indices. Electrostatic descriptors reflect characteristics of the charge distribution of molecules. A detailed description of these descriptors is reported by Todeschini and Consonni.⁴⁵ Finally, we selected two descriptors, GATS4v and BELv7, (numbers 4 and 6 in Table 2) due to the smallest RMSE values in ANFIS modeling of training and testing sets. BELv7 and GATS4v are related to BCUT (Burden CAS-University of Texas eigenvalues) and 2nd autocorrelation descriptors, respectively; both calculated by atomic van der Waals volumes. These descriptors are topochemical indices which quantify information on topology but also specific chemical properties of atoms such as their chemical identity and hybridization state. BCUT descriptors are molecular descriptors defined as eigenvalues of a modified connectivity matrix, which is called the Burden matrix. GATS4v (Geary autocorrelation) is a measure of spatial autocorrelation, which means that adjacent observations of the same phenomenon are correlated. The Geary coefficient is a distance-type function varying from zero to infinity. Strong autocorrelation produces low values of this index; moreover, positive autocorrelation translates into values of between 0 and 1, whereas negative autocorrelation produces values larger than 1.⁴⁵

Results

Development of ANFIS modeling

ANFIS modeling involves two steps: (a) structure identification

Fig. 2 Membership functions for GATS4v as one of the inputs before the training step (up) and after the training step (down) in ANFIS modeling.

and (b) parameter identification. The former is related to finding a suitable number of rules and a proper partition of the feature space. The latter is concerned with the adjustment of system parameters, such as MF (membership function) parameters, linear coefficients and so on. The fuzzy part of ANFIS is mathematically incorporated in the form of membership functions (MFs). This study also presents four Gaussian MF values for each descriptor. For example, Fig. 2 shows two Gaussian membership functions for GATS4v as the first input before and after the ANFIS training step. Totally four rules were used for ANFIS model generation. It is concluded that by increasing the number of MFs per input, the number of rules increases accordingly. The number and type of membership functions were optimized using RMSE as a criterion for the test set. In the training step, different ANFIS models were built using different combinations of six descriptors (in Table 2) by the Leave One Out cross validation method and the simplest final model with only two descriptors (GATS4v and BELv7) was selected according to lowest RMSE value. The two descriptors (GATS4v and BELv7) obtained from different modeling by trial and error were applied to subtractive clustering⁴⁶ which is a fast and one-pass algorithm for estimating the number of clusters and the cluster centers in a set of data. The cluster estimates obtained from a function can be used to initialize iterative optimization-based clustering methods and model identification methods.

We then attempted to investigate the possibility and effectiveness of predicting the descriptor pattern of 8 azaadenine derivatives with an ANFIS approach. In order to find which combination for the training and testing data sets would give better models according to their RMSE values, we aimed to categorize the data for setting up the data fragmentation into training and testing subsets with three approaches. In the first attitude, which was used for the training step, each value in the data set was taken as a test data and the remaining values were

considered for the training data set. Thus, the training data sets contained 44 data; test data sets contained one data each. The data were separated 45 times into train and test data to find out their RMSE values. This method is called the Leave One Out method. Second, the data set contained 45 values, which were sorted according to the descending output value followed by dividing the data set into 10 subsets and the maximum and minimum values in each subset was taken as test data and the remaining values were considered for the training data set. This is a kind of heuristic way of division. Algorithms with the heuristic search strategy are very simple to implement and very fast to produce results. Third, in the randomized method, the parent data set was divided into different subsets of training and test sets so different independent models could be obtained. The training data set contained 25 data and the test data sets contained 20 data each. The experimental ANFIS results of the test set data according to one of the random selections (referred as random-2) are reported in Table 3.

Comparison of the ANFIS model with the RBF and MLR models

For more investigation, the radial basis function (RBF)⁴⁷ and MLR techniques were also used to compare with the ANFIS model in the present work. Different RBF and MLR models were trained using different combinations of six descriptors (Table 2) by the Leave One Out cross validation method. Then, the best models with the lowest RMSE for RBF and MLR were selected. The best models had four descriptors for RBF (numbers 1, 3, 4 and 6 in Table 2) and six descriptors for MLR (numbers 1, 2, 3, 4, 5 and 6 in Table 2), respectively. Then, the

validation of the trained model was examined using different data sets based on randomized data selection. The results of the experimental pK_i and the predicted pK_i for the test set of random-3 selection in the two-descriptor ANFIS final model (GATS4v and BELv7), the four-descriptor (GATS4v, BELv7, R3p and More25m) RBF final model and the six-descriptor (GATS4v, BELv7, R3p, Dp, More25m and MATS7v) MLR model are presented in Fig. 3. Giorgi *et al.*³⁸ already reported a five-parameter MLR model with different descriptors analyzed by the CODESSA program and the statistical parameter ($R^2 = 0.6812$ and $Q^2 = 0.5787$) comparative to the MLR model presented in this work. The results of RMSE and Q^2 of Leave One Out, heuristic and randomized methods for ANFIS, RBF and MLR models are summarized in Table 4.

Among the three approaches for the train and test set, it seems that the Leave One Out and randomized method outperformed the other method with RMSE values of 0.331 for Leave One Out, 0.575 for heuristic and 0.440 for the randomized methods and with Q^2 values of 0.733 for Leave One Out, 0.237 for heuristic and 0.504 for the randomized method in the test data set. It is clear from this table that the results of RMSE and Q^2 for the ANFIS model is superior compared with those of the MLR and RBF, besides that the ANFIS model uses fewer descriptors for modeling; consequently, a simpler model will be generated.

Conclusion

In this study, we investigated the possibility and effectiveness of predicting the descriptor pattern of 8-azaadenine derivatives with the adaptive neuro-fuzzy inference system (ANFIS); and satisfactory results were obtained. We applied the genetic

Table 3 ANFIS results of testing data according to random-2 selection

No.	Compound	GATS4v	BELv7	Observed pK_i	Predicted pK_i
1	A9	0.805	1.286	2.7959	2.2927
2	B1	0.97	1.006	0.719	0.49207
3	C10	0.842	1.05	1.2676	1.5961
4	C13	0.816	1.286	2.7328	2.2735
5	C1	0.88	1.006	1.6021	0.91539
6	B4	0.883	1.2	1.1024	1.5534
7	A11	0.755	1.187	2.1249	2.1445
8	C8	0.711	1.203	2.1739	2.1769
9	B11	0.825	1.187	1.4949	2.05
10	D6	0.966	1.286	2.1079	1.6952
11	A5	0.833	1.244	1.8794	2.1362
12	C4	0.821	1.201	2.3665	2.1001
13	D1	1.02	1.197	1.8268	1.2885
14	D4	0.934	1.291	2.1871	1.7316
15	B9	0.8	1.195	1.4815	2.1399
16	A8	0.817	1.201	1.9586	2.1147
17	C11	0.758	1.187	2.3188	2.1442
18	A13	0.813	1.286	2.5686	2.2799
19	A8	0.83	1.198	1.9586	2.0486
20	A2	0.852	1.006	1.042	1.3808

Fig. 3 Results of experimental pK_i and predicted pK_i for the test set of random-3 selection in the two-descriptor (GATS4v and BELv7) ANFIS final model and the four-descriptor (GATS4v, BELv7, R3p and More25m) RBF final model, the six-descriptor (GATS4v, BELv7, R3p, Dp, More25m and MATS7v) MLR model.

Table 4 Statistical parameters of test sets in the heuristic, randomized and Leave One Out methods for the ANFIS, RBF and MLR models

Method	Leave One Out		Heuristic		Random-1		Random-2		Random-3	
	RMSE	Q^2	RMSE	Q^2	RMSE	Q^2	RMSE	Q^2	RMSE	Q^2
ANFIS	0.331	0.733	0.575	0.237	0.483	0.452	0.445	0.563	0.394	0.498
RBF	0.609	0.148	0.690	-0.061	0.535	0.332	0.731	-0.156	0.728	-0.715
MLR	0.635	0.083	0.658	0.039	0.572	0.229	0.842	-0.442	0.455	0.353

algorithm and stepwise regression technique for selecting obtained descriptors, followed by the ANFIS approach for modeling the descriptors. These descriptors were derived from either two- or three-dimensional molecular structure indices based on BCUT and 2nd autocorrelation, 3D-MoRSE, WHIM, and GETAWAY descriptors. Using the two most relevant descriptors (GATS4v and BELv7) for ANFIS, and four descriptors for the RBF and six descriptors for the MLR model, we compared the ANFIS results with RBF and MLR models in randomized method. These results suggest that a comprehensive QSAR model of pK_i of 8-azaadenine derivatives may be built by ANFIS using only two descriptors with better RMSE and Q^2 , compared to the four-descriptor RBF model and the six-descriptor MLR model. New predictive ANFIS modeling can be expected to be useful for predicting affinity properties in drug design and experimental testing.

References

1. H. Kubinyi and O. H. Kehrhan, *Arzneim.-Forsch Drug. Res.*, **1978**, 28, 598.
2. M. Fernandez and J. Caballero, *J. Bioorg. Med. Chem.*, **2006**, 14, 280.
3. I. Valkova, M. Vracko, and S. C. Basak, *Anal. Chim. Acta*, **2004**, 509, 179.
4. D. Weekes and G. B. Fogel, *Biosystems*, **2003**, 72, 149.
5. G. Schneider and P. Wrede, *Prog. Biophys. Mol. Biol.*, **1998**, 70, 175.
6. D. T. Manallack, D. D. Ellis, and D. J. Livingstone, *J. Med. Chem.*, **1994**, 37, 3758.
7. G. Gini, M. Lorenzini, E. Benfenati, P. Grasso, and M. Bruschi, *J. Chem. Inf. Comput. Sci.*, **1999**, 39, 1076.
8. S. S. So and M. Karplus, *J. Med. Chem.*, **1996**, 39, 5246.
9. T. E. Exner and J. Brickmann, *J. Mol. Model*, **1997**, 3, 321.
10. T. E. Exner, M. Keil, and J. Brickmann, "Fuzzy Set Theory and Fuzzy Logic and Its Application to Molecular Recognition", **2003**, Wiley-VCH, Weinheim, 1216.
11. J. S. R. Jang, *IEEE Trans. Neural Networks*, **1992**, 3, 714.
12. J. S. R. Jang, *IEEE Trans. Syst. Man Cybern.*, **1993**, 23, 665.
13. R. Fuller, "Introduction to Neuro-Fuzzy Systems, *Advances in Soft Computing Series*", **1999**, Springer-Verlag, Berlin/Heidelberg, Heidelberg, 289.
14. E. Buyukbingol, A. Sisman, M. Akyildiz, F. N. Alparslan, and A. Adejare, *Bioorg. Med. Chem.*, **2007**, 15, 4265.
15. M. Jalali-Heravi and M. Asadollahi-Baboli, *Eur. J. Med. Chem.*, **2009**, 44, 1463.
16. M. Jalali-Heravi, M. Asadollahi-Baboli, and A. Mani-Varnosfaderani, *J. Pharm. Biomed. Anal.*, **2009**, 50, 853.
17. A. Khajeh and H. Modarress, *J. Hazard. Mater.*, **2010**, 179, 715.
18. R. E. Shaffer and G. W. Small, *Anal. Chem.*, **1997**, 69, 236A.
19. D. R. Rogers and A. J. Hopfinger, *J. Chem. Inform. Comput. Sci.*, **1994**, 34, 854.
20. B. T. Luke, *J. Chem. Inf. Comput. Sci.*, **1994**, 34, 1279.
21. J. M. Sutter, S. L. Dixon, and P. C. Jurs, *J. Chem. Inf. Comput. Sci.*, **1995**, 35, 77.
22. A. Herro and M. C. Oritz, *Anal. Chim. Acta*, **1999**, 378, 245.
23. A. S. Bangalore, R. E. Shaffer, G. W. Small, and M. A. Arnold, *Anal. Chem.*, **1996**, 68, 4200.
24. Q. Ding, G. W. Small, and M. A. Arnold, *Anal. Chem.*, **1998**, 70, 4472.
25. B. B. Fredholm, A. P. Ijzerman, K. A. Jacobson, K. N. Klotz, and J. Linden, *Pharmacol. Rev.*, **2001**, 53, 527.
26. V. Ralevic and G. Burnstock, *Pharmacol. Rev.*, **1998**, 50, 413.
27. C. E. Müller, *Expert Opin. Ther. Pat.*, **1997**, 7, 419.
28. J. A. Ribeiro, A. M. Sebastiao, and A. de Mendoca, *Prog. Neurobiol.*, **2003**, 68, 377.
29. K. Dalla, J. C. Shiryock, R. Shreeniwas, and L. Belardinelli, *Curr. Top. Med. Chem.*, **2003**, 32, 369.
30. K. N. Klotz, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **2000**, 362, 382.
31. G. Biagi, I. Giorgi, O. Livi, V. Scartoni, C. Breschi, C. Martini, and R. Scatizzi, *Farmaco*, **1995**, 50, 659.
32. G. Biagi, I. Giorgi, O. Livi, V. Scartoni, A. Lucacchini, C. Martini, and P. Tacchi, *Farmaco*, **1994**, 49, 93.
33. G. Biagi, I. Giorgi, O. Livi, V. Scartoni, A. Lucacchini, C. Martini and P. Tacchi, *Farmaco*, **1995**, 50, 13.
34. G. Biagi, I. Giorgi, O. Livi, V. Scartoni, A. Lucacchini, C. Martini, and P. Tacchi, *Farmaco*, **1994**, 49, 187.
35. G. Biagi, I. Giorgi, O. Livi, V. Scartoni, and A. Lucacchini, *Farmaco*, **1996**, 51, 395.
36. G. Biagi, I. Giorgi, M. Leonardi, O. Livi, F. Pacchini, V. Scartoni, B. Costa, and A. Lucacchini, *Eur. J. Med. Chem.*, **2003**, 38, 801.
37. G. Biagi, I. Giorgi, A. Nardi, F. Pacchini, V. Scartoni, and A. Lucacchini, *Eur. J. Med. Chem.*, **2003**, 38, 983.
38. I. Giorgi, A. M. Bianucci, G. Biagi, O. Livi, V. Scartoni, M. Leonardi, D. Pietra, A. Coi, I. Massarelli, F. A. Nofal, F. L. Fiamingo, P. Anastasi, and G. Giannini, *Eur. J. Med. Chem.*, **2007**, 42, 1.
39. M. P. Gonzalez, C. Teran, M. Teijeirac, and P. Besadac, *Bioorg. Med. Chem. Lett.*, **2005**, 15, 2641.
40. M. P. Gonzalez, M. del Carmen, and T. Moldes, *Bioorg. Med. Chem.*, **2004**, 12, 2985.
41. H. Takagi and M. Sugeno, *IEEE Trans. Syst. Man Cybern.*, **1985**, 15, 116.
42. M. Setnes, R. Babuska, and H. B. Verbruggen, *Int. J. Human Comp. Study*, **1998**, 49, 159.
43. J. S. R. Jang, C. T. Sun, and E. Mizutani, "Neuro-Fuzzy and Soft Computing: A Computational Approach to Learning and Machine Intelligence", **1997**, Prentice-Hall International Ltd., London, 614.
44. H. Takagi and M. Sugeno, in Proceedings of IFAC Symposium on Fuzzy Information, Knowledge Representation and Decision Analysis, **1983**, 55.
45. R. Todeschini and V. Consonni, "Handbook of Molecular Descriptors", **2000**, Wiley/VCH, Weinheim.
46. S. Chiu, *J. Intel. Fuzzy Syst.*, **1994**, 2, 267.
47. C. Jareanpon, N. Eua-Anant, C. Vithsupalert, and W. Pensuwon, the 3rd International Symposium on Communications and Information Technologies, **2003**, 621.