Eur J Med Chem (1995) 30, 569–574

Artificial neural network studies in quantitative structure–activity relationships of antifungal azoxy compounds

K Hasegawa[1], T Deushi[1], O Yaegashi[2], Y Miyashita[2]*, S Sasaki[2]

[1]*Tokyo Research Laboratories, Kowa Co Ltd, 17-43-2, Noguchi-cho Higashimurayama, Tokyo, 189;*
[2]*Department of Knowledge-Based Information Engineering, Toyohashi University of Technology, Tempaku-cho, Toyohashi, 441, Japan*

(Received 5 January 1995; accepted 22 March 1995)

Summary — Artificial neural networks (ANN) based on the back-propagation algorithm (BP algorithm) were applied to a quantitative structure–activity relationship (QSAR) study for 30 azoxy compounds with antifungal activity. The ANN model could well explain the variance of the antifungal activity owing to its ability to deal with a nonlinear tendency in the data set. A modified BP algorithm proposed by the authors has provided the ANN model with a more enhanced predictive capability. Finally, a transformation of the final ANN model to a polynomial of original physico-chemical parameters was shown to be useful to elucidate the structural requirements for the antifungal activity.

artificial neural network / back-propagation algorithm / quantitative structure–activity relationship / azoxy compound / antifungal activity

Introduction

For medicinal chemists, a quantitative structure–activity relationships (QSAR) study is an important task in the development of new agents because it can keep the number of synthesized and tested compounds to a minimum [1]. QSAR relates the variation in biological activity to the variation in chemical structure for a series of compounds. It is more useful to understand how a compound reacts with its target receptor by comparison of QSAR models with molecular graphics if the X-ray crystal structure of the compound bound to the receptor is available [2].

A large number of successful works using QSAR techniques have emerged since Hansch and coworkers [3] reported the first multivariate approach. This approach, called the Hansch–Fujita method, employs multiple linear regression (MLR) and can give a quantitative prediction of the biological activity by the QSAR equation. In the case where the biological activity is defined as a class category, pattern recognition methods [4, 5], such as the SIMCA (soft independent modeling of class analogy) and KNN (K-nearest neighbors) methods, are useful for classifying biological compounds. Wold [6] proposed a novel multivariate method of partial least squares (PLS) regression for QSAR studies. PLS can be regarded as a generalization of MLR and give more robust linear QSAR model equations. Successful PLS modelings of QSAR for thiolcarbamates [7] and endothelin-1 analogs [8] have been reported. Cramer [9] introduced the CoMFA (comparative molecular field analysis) approach based on PLS regression for 3D-QSAR modeling.

However, in some applications, the desired QSAR model is not a linear function of physicochemical parameters describing the chemical structures. This means that the standard linear MLR and PLS modelings are suboptimal or even useless methods in this situation. Attempts to include the quadratic and cross-product terms of physico-chemical parameters to the model equation were unsuccessful at improving QSAR models [10].

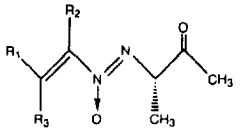
Artificial neural networks (ANN) are new information processing systems which simulate the function of the brain. The use of ANN in QSAR studies is now a standard procedure. Andrea *et al* [10] found that the ANN model of triazine derivatives as dihydrofolate reductase (DHFR) inhibitors led to enhanced fittings and predictions relative to the conventional MLR method. Richards *et al* [11] have studied QSAR of

*Correspondence and reprints

pyrimidine derivatives as DHFR inhibitors with ANN and suggested that ANN can outperform the conventional MLR model.

In this article QSAR modeling of azoxy compounds with antifungal activity was investigated using ANN. First, a data set of the antifungal activity was examined by the PLS method to verify a nonlinear tendency. Next, ANN with a back-propagation algorithm (BP algorithm) was applied to this data set and the resulting model was improved in both calibration and validation steps compared with the PLS model. It was then demonstrated that the modified BP algorithm by which the variance of the connection weights was decreased in the training phase resulted in the ANN model with a more enhanced predictive capability. Finally, the transformation of the ANN model to a polynomial of original physico-chemical parameters was shown to be useful to elucidate the structural requirements of the antifungal activity.

Table I. Chemical structures of azoxy compounds and observed antifungal activity.



Compound	R_1	R_2	R_3	*log(1/MIC)*
1	CH_3	H	H	4.10
2	C_2H_5	H	H	5.04
3	n-C_3H_7	H	H	5.06
4	n-C_4H_9	H	H	5.39
5	n-C_5H_{11}	H	H	6.00
6	$CH{=}CH_2$	H	H	4.12
7	C_6H_5	H	H	4.85
8	$CH_2C_6H_5$	H	H	5.77
9	n-C_4H_9	CH_3	H	6.30
10	n-C_4H_9	n-C_5H_{11}	H	4.93
11	H	C_6H_5	H	4.54
12	SCH_3	C_6H_5	H	4.91
13	4-$NO_2C_6H_4$	H	H	3.72
14	$(CH_2)_2C_6H_5$	H	H	5.20
15	$(CH_2)_2C_6H_5$	CH_3	H	5.51
16	n-C_4H_9	4-ClC_6H_4	H	6.22
17	3-ClC_6H_4	H	CH_3	5.52
18	4-$OCH_3C_6H_4$	H	CH_3	5.21
19	4-$C_6H_5C_6H_4$	H	CH_3	5.87
20	2-Thienyl	H	CH_3	5.47
21	C_6H_5	H	C_2H_5	5.79
22	C_6H_5	H	n-C_3H_7	6.10
23	C_6H_5	H	n-C_4H_9	5.82
24	4-ClC_6H_4	H	n-C_4H_9	5.59
25	C_6H_5	H	CF_3	4.65
26	SCH_3	H	SCH_3	3.67
27	4-ClC_6H_4	CH_3	CH_3	6.15
28	4-ClC_6H_4	n-C_5H_{11}	CH_3	4.73
29	H	4-ClC_6H_4	n-C_4H_9	5.89
30	H	C_6H_5	Cl	5.21

Materials and methods

Data set

The data set used in this study is the antifungal activity of 30 azoxy compounds in three substituent positions (R_1, R_2 and R_3). Of these, 16 azoxy compounds in two substituent positions (R_1 and R_2) have been previously studied by PLS and a significant PLS model was obtained [12]. The antifungal activity is expressed as a logarithm of the reciprocal of the MIC (minimum inhibitory concentration) against *Trichophyton rubrum*. The chemical structures and the antifungal activity of 30 azoxy compounds are shown in table I.

The structural descriptor describing each chemical structure consists of a nine-dimensional vector **X**.

$$\mathbf{X} = (Z_1(R_1), Z_2(R_1), Z_3(R_1), Z_1(R_2), Z_2(R_2), Z_3(R_2), Z_1(R_3), Z_2(R_3), Z_3(R_3)) \quad [1]$$

The elements (physico-chemical parameters) of the vector **X** are three principal properties PPs, ($Z_1(R_1)$, $Z_2(R_1)$, $Z_3(R_1)$) for the substituent R_1, three PPs ($Z_1(R_2)$, $Z_2(R_2)$, $Z_3(R_2)$) for the substituent R_2 and three PPs ($Z_1(R_3)$, $Z_2(R_3)$, $Z_3(R_3)$) for the substituent R_3. These PPs are the same as those used in the previous PLS study [12] and most PPs values have been quoted from the article of Clementi [13]. Three PP values are the first, second and third principal component scores of physico-chemical parameters of 100 aromatic substituents. The first PP mainly represents steric bulkiness and hydrophobicity. The second PP and third PP mainly represent electronic property and hydrophobicity, respectively.

The PP values of six substituents are unknown: 4-$NO_2C_6H_4$, $(CH_2)_2C_6H_5$, 4ClC_6H_4, 3-ClC_6H_4, 4-$OCH_3C_6H_4$ and 4-$C_6H_5C_6H_4$. These PP values were calculated by the same procedures as those made in the previous PLS study [12]. Table II lists the PP values of all the substituents used in this study including the computed ones.

PLS method

Because the main purpose of this study is the application of ANN to QSAR data, only a brief description of the PLS method will be given here [14]. The PLS model is a principal component-like model and gives the relationships between independent variables X and a dependent variable y using latent variables t_a.

$$X = X_0 + \sum_{a=1}^{A} t_a p_a' + E \quad [2a]$$

Table II. Principal properties used as structural descriptors for ANN.

Substituent	Z_1	Z_2	Z_3
H	0.000	0.000	0.000
CH_3	1.220	−0.213	0.816
C_2H_5	2.275	−0.326	0.796
n-C_3H_7	3.203	−0.425	0.817
n-C_4H_9	4.298	−0.452	0.846
n-C_5H_{11}	5.231	−0.526	0.871
$CH{=}CH_2$	2.367	−0.944	0.593
C_6H_5	4.572	−1.398	1.099
$CH_2C_6H_5$	3.021	−0.552	2.501
SCH_3	2.381	−1.066	0.706
2-Thienyl	4.237	−1.489	1.021
CF_3	1.303	−2.352	1.364
Cl	1.367	−1.683	0.743
4-$NO_2C_6H_4$	5.614	−2.216	0.560
$(CH_2)_2C_6H_5$	6.367	−1.273	0.597
4-ClC_6H_4	5.913	−1.404	0.957
3-ClC_6H_4	4.964	−1.798	1.506
4-$OCH_3C_6H_4$	5.845	−1.575	0.782
4-$C_6H_5C_6H_4$	8.774	−1.731	0.864

$$y = y_0 + \sum_{a=1}^{A} t_a q_a + f \qquad [2b]$$

where X_0 and y_0 are the corresponding mean value matrix and vector, respectively. p_a and q_a are the ath loadings for X and y, respectively. p_a' is the transpose of a vector p_a. E and f are residuals. A is the number of components for the PLS model determined by cross-validation [15]. The latent variable t_a is expressed as a linear combination of independent variables X and the PLS weight w_a.

$$t_a = (X - X_0 - \sum_{a=1}^{A-1} t_a p_a')w_a \qquad [3]$$

If eq [3] is substituted into eq [2b], then a MLR-like model equation is obtained:

$$y = y_0 + (X - X_0)b + f \qquad [4]$$

where b is a coefficient vector for the PLS model. This model can provide insight in terms of original independent variables X. In this study, y is the antifungal activity. X is 9 PPs for the three substituents R_1, R_2 and R_3.

Artificial neural network (ANN)

ANN are computer-modeled systems containing a number of nodes which are connected into net-like structure. In the present study, a three-layer backpropagation network was employed for QSAR modeling. The network consists of input, hidden and output layer nodes which are schematically drawn as circles and connected by bonds with connection weights as shown in figure 1. Each input layer node obtains an input signal or independent variable X_p; a principal property Z. Similarly, the output layer node produces a calculated dependent variable y; the antifungal activity. Each of the input and hidden layers has an additional bias node for accommodating nonzero offset in the modeling. The bias node obtains a signal with an intensity 1.0 and distributes the signal to the next layer.

If input signals are given in the input layer, the input nodes only serve as distributors of input signals to the hidden layer. Next, the net input s_a *is* calculated by eq [5a] in the ath hidden node and it is transformed by the sigmoidal function $f(s_a)$ by eq [5b] to give the output t_a of the hidden node.

$$s_a = \sum_{p=1}^{d} W_{ap} X_p + \theta_a \qquad [5a]$$

$$t_a = f(s_a) = (1 + \exp(-s_a/T))^{-1} \qquad [5b]$$

where d is the number of input nodes and W_{ap} is the connection weight between hidden node a and input node p. θ_a is the bias connecting hidden node a to the input layer bias node. T is an adjustable parameter determining the shape of the sigmoidal curve and referred to as a computational temperature. Finally, the output node produces a calculated dependent variable y by a linear equation.

$$y = \sum_{a=1}^{A} V_a t_a + \theta = \sum_{a=1}^{A} V_a f(s_a) + \theta \qquad [6]$$

where V_a is the connection weight between the output node and hidden node a. θ is the bias connecting the output node to the hidden layer bias node. In the present study, we have not employed the sigmoidal transformation in the output node because of simplification of the ANN model and omission of rescaling the final output of the network.

The BP algorithm has been widely used in the ANN applications [16] and this algorithm was employed in this study. The BP algorithm is based on a gradient descent method to minimize an error function E with respect to the connection weights (W_{ap}, V_a) and biases (θ_a, θ) from the top output layer toward the bottom input layer. E is given as follows:

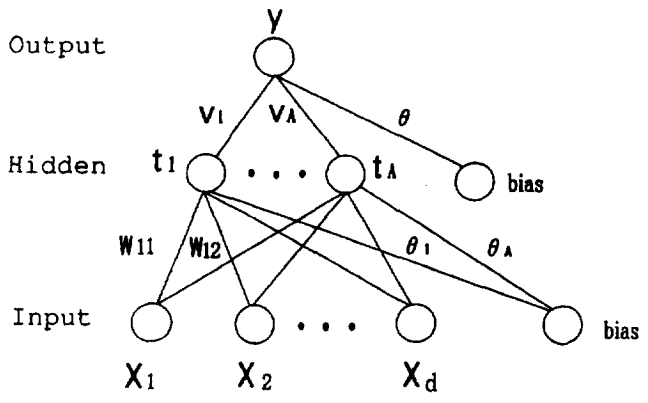


Fig 1. Three-layer neural networks.

$$E = 1/2 \sum_{i=1}^{N} E_i^2 = 1/2 \sum_{i=1}^{N} [y_i\,(\text{obs}) - y_i\,(\text{calc})]^2 \qquad [7]$$

where N is the number of objects in the training set and E_i represents the error of the ith training data. y_i (calc) is the calculated output value for ith training data from the output node and y_i (obs) is its true (observed) value. In the gradient descent method, the partial derivatives of the error function E_i are utilized and the connection weights V_a and W_{ak} are iteratively changed from their initially assigned random values.

$$\Delta V_a(n+1) = V_a(n+1) - V_a(n) = -\alpha \cdot \partial E_i / \partial V_a \qquad [8a]$$

$$\Delta W_{ap}(n+1) = W_{ap}(n+1) - W_{ap}(n) = -\alpha \cdot \partial E_i / \partial W_{ap} \qquad [8b]$$

where n and $n + 1$ represents the number of iterations in the training phase. α is a parameter, which determines the learning rate. Similar equations are used for adjusting the biases θ_a and θ, and β is the corresponding learning rate. In order to prevent sudden changes in the direction in which corrections are made, a momentum coefficient (mc) can be incorporated into eqs [8a] and [8b] [17].

$$\Delta V_a(n+1) = -\alpha \cdot \partial E_i / \partial V \alpha - \text{mc} \cdot \Delta V_a(n) \qquad [9a]$$

$$\Delta W_{ap}(n+1) = -\alpha \cdot \partial E_i / \partial W_{ap} - \text{mc} \cdot \Delta W_{ap}(n) \qquad [9b]$$

It has been said that even if simple linear data are given, ANN tend to produce a more complicated model due to meaningless growth of the connection weights [17]. This tendency has no advantage in the prediction of the antifungal activity for unsynthesized compounds. In order to reduce this tendency as much as possible, eqs [9a] and [9b] were modified in the following manner.

$$V_a(n+1) = V_a(n) + \Delta V_a(n+1) - \gamma \cdot V_a(n+1) \qquad [10a]$$

$$W_{ap}(n+1) = W_{ap}(n) + \Delta W_{ap}(n+1) - \gamma \cdot W_{ap}(n+1) \qquad [10b]$$

where γ is a small value and represents a parameter which suppresses the variance of the connection weights. In the following section we will demonstrate that modified BP algorithm (eqs [10a] and [10b]) provides a more predictive model than the model derived from the conventional BP algorithm (eqs [9a] and [9b]).

Results and discussion

PLS analysis

PLS analysis was carried out using the Unscrambler software package developed by Martens [14] on an IBM PS/2 microcomputer. The antifungal activity and 9 PPs were mean-centered. A five-component PLS model was derived by the leave-one-out procedure. Converting the PLS model to the MLR-like model (eq [4]), the following equation was obtained.

$$\begin{aligned}\log(1/\text{MIC}) = {} & 0.361Z_1(\text{R}_1) + 1.221Z_2(\text{R}_1) + 0.688Z_3(\text{R}_1) \\ & - 0.179Z_1(\text{R}_2) - 0.628Z_2(\text{R}_2) \\ & + 0.598Z_3(\text{R}_2) + 0.239Z_1(\text{R}_3) \\ & + 0.134Z_2(\text{R}_3) + 0.239Z_3(\text{R}_3) + 3.960\end{aligned}$$

$n = 30, A = 5, s = 0.426, r = 0.844, r_{\text{pred}} = 0.666$

where s, r and r_{pred} are the standard error, conventional correlation coefficient and predictive coefficient, respectively. The standard deviation is rather large and the r value is not enough to explain the variance of the antifungal activity. A relatively low r_{pred} value indicates that the PLS model is not predictive. This means that the antifungal activity data would have a nonlinear tendency and may not be explained by the linear PLS model.

ANN analysis

The ANN experiments were simulated using a program written in Fortran language and run on a VAX station. PP values and the antifungal activity were mean-centered before the training of the network. In the ANN experiments, the number of iterations for the training of the network is determined so that it gives the highest predictive correlation coefficient r_{pred} by leave-one-out procedure.

The number of nodes in the hidden layer is an important factor determining the network's performance. That is, too many nodes cause the network to memorize the data set (overfitting). The networks with few nodes may be insufficient to use all the information from the data set (underfitting). It is desirable to construct the network which generalizes the patterns of the data set rather than merely memorizes them [10]. In order to determine the number of hidden nodes, we have changed the number of hidden nodes and observed the conventional and predictive correlation coefficients while holding all other parameters constant ($\alpha = 0.1$, $\beta = 0.1$, mc = 0.1 and $T = 1.0$). Five sets of randomly assigned connection weights and biases were used to estimate the correlation coefficients corresponding to the global minimum of the network. The best results of the network with different number of hidden nodes are shown in figure 2. A filled dot and circle represent the correlation coefficient r and predictive one r_{pred} against the number of hidden nodes, respectively. The best predictive performance r_{pred} was obtained with three hidden nodes. We have concluded that the ANN model with three hidden nodes would be the most predictive and optimal. Therefore, the architecture of the network was decided to be 9-3-1 (input-hidden-output).

In order to determine the optimal values of learning rates α and β in the 9-3-1 network, α and β were varied. Both learning rates of 0.03 gave a better calibration and validation (r = 0.989, r_{pred} = 0.802) models. Because other parameters (mc = 0.1 and T = 1.0) had little effect on the modeling performance, they were held constant in the final ANN experiments.

Finally, ANN with modified BP algorithm (eqs [10a] and [10b]) in the 9-3-1 network were applied to this data set. A training cycle, in which modified BP

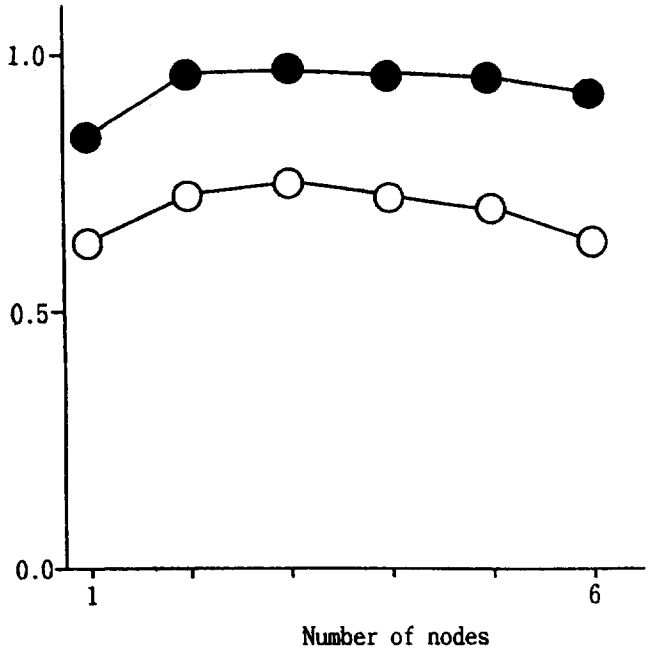


Fig 2. Number of nodes in hidden layer against conventional and predictive correlation coefficients. Black: r; white: r_{pred}.

algorithm was applied to the network (γ = 0.0001) every four training iterations, was employed. The training of the network by the repeat cycles provided the ANN model with a more enhanced predictive capability (r = 0.984, r_{pred} = 0.814). This increased predictive capability of the ANN model has also been verified in the external validation of ^{13}C NMR chemical shift of halomethanes (Miyashita *et al*, unpublished results). Modified BP algorithm may be useful in order to overcome several shortcomings (overfitting and meaningless growth of the connection weights) of ANN. Figure 3 shows the plot of calculated *versus* observed antifungal activity by the final ANN model.

Transformation of the ANN model

The ANN model is difficult to interpret because it consists of some hidden nodes which produce nonlinear outputs for describing the nonlinear behavior in the data set. The transformation of the ANN model to a polynomial of original physicochemical parameters (eq [11]) is necessary to elucidate the structural requirements for the antifungal activity.

$$y = C_0 + \Sigma C_p X_p + \Sigma\Sigma C_{pq} X_p X_q + \Sigma\Sigma\Sigma C_{pqr} X_p X_q X_r + \ldots \quad [11]$$

where C_0, C_p, C_{pq} and C_{pqr} are the coefficients of the constant, first, second and third order terms, respectively.

We have investigated how to represent the ANN model with a polynomial. The details of the investigation will be published and the important results will be described. The transformation procedure from the ANN model eq [6] to the polynomial (eq [11]) is as follows. The Taylor expansion for $f(s_a)$ (eq [5b]) about the point $s_a = b$ is substituted in eq [6].

$$\begin{aligned} y &= \theta + \Sigma V_a(f(b) + f^{(1)}(b)\,(s_a - b) + f^{(2)}(b)(s_a - b)^2/2! \\ &\quad + f^{(3)}(b)(s_a - b)^3/3! + \ldots) \\ &= \theta + \Sigma V_a(f(b) + f^{(1)}(b)\,(\Sigma W_{ap} X_k + \theta_a - b) \\ &\quad + f^{(2)}(b)\,(\Sigma W_{ap} X_k + \theta_a - b)^2/2! + \ldots) \end{aligned} \quad [12]$$

The resulting equation is compared with eq [11] and then the coefficients of zero, first, second and third order term coefficients are calculated in terms of the connection weights and biases.

$$C_0 = \theta + \Sigma V_a f(\theta_a) \quad [13a]$$
$$C_p = \Sigma V_a W_{pa} f^{(1)}(\theta_a) \quad [13b]$$
$$C_{pp} = \Sigma V_a W_{pa}^2 f^{(2)}(\theta_a)/2! \quad [13c]$$
$$C_{pq} = \Sigma V_a W_{pa} W_{qa} f^{(2)}(\theta_a),\ (p \neq q) \quad [13d]$$
$$C_{ppp} = \Sigma V_a W_{pa}^3 f^{(3)}(\theta_a)/3! \quad [13e]$$
$$C_{ppq} = \Sigma V_a W_{pa}^2 W_{qa} f^{(3)}(\theta_a)/(2!\,1!) \quad [13f]$$
$$C_{pqr} = \Sigma V_a W_{pa} W_{qa} W_{ra} f^{(3)}(\theta_a),\ (p \neq q \neq r) \quad [13g]$$

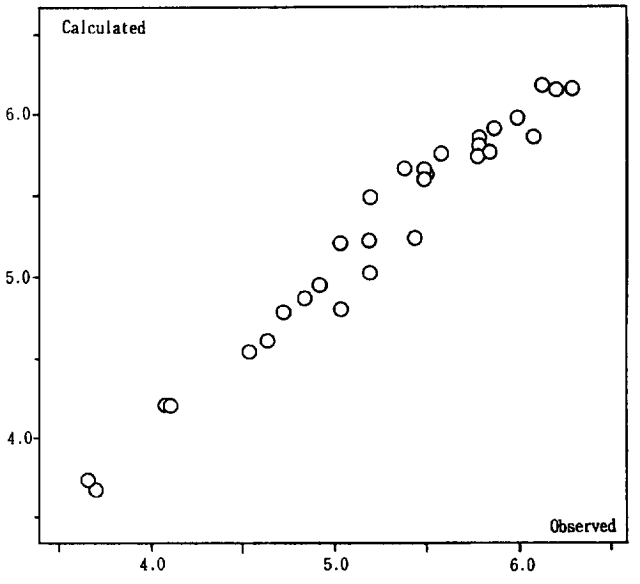


Fig 3. Plot of calculated against observed antifungal activity by final ANN model.

Table III. Coefficients of the ANN model and PLS model[a].

	ANN Model	*PLS Model*
$Z_1(R_1)$	0.785	0.346
$Z_2(R_1)$	1.479	1.122
$Z_3(R_1)$	1.111	0.742
$Z_1(R_2)$	–0.542	–0.419
$Z_2(R_2)$	–2.042	–1.447
$Z_3(R_2)$	0.385	1.015
$Z_1(R_3)$	0.231	0.204
$Z_2(R_3)$	0.799	0.485
$Z_3(R_3)$	0.707	0.623

[a]Coefficients represented by the mean-centered physicochemical parameters.

Table III lists the C_p values of the final ANN model derived from modified BP algorithm in the 9-3-1 network. Table III also lists the values of the coefficients of the MLR-like PLS model (eq [11]). It is noted that the first order term coefficients for ANN and MLR-like models have the same sign. This shows that the final ANN model has a reasonable rule between the physicochemical parameters and the antifungal activity in the linear portion. The linear portion of the final ANN model roughly suggests that potent azoxy compounds can be produced by increasing steric bulkiness and hydrophobicity of the two substituents R_1 and R_3 and by increasing electron-withdrawing ability of the substituent R_2. The structural requirements for azoxy compounds with three substituents are consistent with our results for azoxy compounds with two substituents (R_1 and R_2) (Miyashita *et al*, unpublished results).

Conclusion

In the present study, we have developed the ANN model of azoxy compounds with antifungal activity by the BP algorithm. This model could well explain the variance of the antifungal activity in which the nonlinear tendency existed. We have also demonstrated that modified BP algorithm proposed by our group has provided the ANN model with a more enhanced predictive capability. Moreover, the transformation of the final ANN model to the polynomial function of original physicochemical parameters was shown to be useful to elucidate the structural requirements of the antifungal activity.

Acknowledgments

The authors thank the Ishida Foundation and the Ministry of Education, Science and Culture under Grant-in-Aid for Scientific Research for financial support.

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