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ORIGINAL PAPER

Prediction of antibacterial activity of pleuromutilin derivatives by genetic algorithm–multiple linear regression (GA–MLR)

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Abstract Use of quantitative structure–activity relationships for prediction of the antibacterial activity of pleuromutilin derivatives was studied. A suitable set of molecular descriptors was calculated and the important descriptors were selected by using the variable selections of stepwise multiple linear regression and genetic algorithm. Principal-components analysis was used to select the training set. The models were validated by use of leave-one-out (LOO) cross-validation, external test set, and the *Y*-randomization test. Comparison of the results obtained revealed the superiority of the genetic algorithm over the stepwise multiple regression method for feature selection. One genetic algorithm–multiple linear regression (GA–MLR) model with six selected descriptors was obtained. The root mean square errors of the training and test sets for the GA–MLR model were calculated to be 0.423 and 0.523, and the correlation coefficients were 0.839 and 0.807. The statistical parameter of LOO cross validation test correlation coefficients on the GA–MLR model was 0.760. The predictive ability of the model was satisfactory and it can be used for designing similar groups of antibacterial compounds.

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Introduction

Antibacterial resistance by hospital-acquired Gram-positive bacterial pathogens, for example methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE), has become a serious medical problem [1, 2]. Although antibacterial agents including linezolid, quinupristin/dalfopristin, and teicoplanin are now available for treatment of infections caused by resistant bacteria, these agents produce undesirable side effects and their efficacy is restricted because of the development of resistant mutants. To combat such drug-resistant bacterial strains, there is an increasing need to discover and develop novel classes of antibiotics, particularly agents with new mechanisms of action and, consequently, no cross-resistance to marketed antibacterial agents. Recently, novel antibiotic pleuromutilin analogues have been reported by Hirokawa et al. [3–5].

Although several experimental methods are available for screening the estrogenic activity of chemicals (e.g., in-vivo and in-vitro assay tests), and these all have also been carried out using receptors and other biological materials of human, rat, mouse, and calf origin, at least [6], they are costly, time-consuming, and can potentially produce toxic side products from the experimental methods used today. An efficient way of obtaining a complete set of the data without the need to perform expensive laboratory experiments is application of quantitative structure–activity relationship (QSAR) techniques. A QSAR model describes a mathematical relationship between the structural attribute(s) and activity of a set of chemicals. The potential promise of using QSAR models for screening of chemical databases or virtual libraries before their synthesis seems equally attractive to chemical manufacturers, pharmaceutical companies, and government agencies, particularly in times of shrinking resources [7]. Many different modeling technologies, for example multiple linear regression (MLR), partial least squares (PLS), and different types of artificial neural networks (ANN) have been widely used in QSAR modeling [8–10]. The application of these techniques usually requires variable selection for building well-fitted models. In this work, we employed the stepwise (SW) and the genetic algorithm (GA) selection method for the variable selection in the MLR method. The main objective of this work was to establish a new QSAR model for predicting the antibacterial activity of novel pleuromutilin derivatives using the GA–MLR technique. The performance of this model was compared with that of the SW–MLR method.

Results and discussion

Principal-components analysis (PCA) was performed with the calculated structure descriptors for the whole data set to detect the homogeneities in the data set and to show spatial location of samples to assist separation of the data into training and test sets. The PCA results show that two principal components (PC1 and PC2) describe 58.60% of the overall variables, as follows: PC1 = 34.93%, PC2 = 23.67%. Because almost all variables can be accounted for by the first two PCs, their score plot is a reliable representation of the spatial distribution of the points for the data set. The plot of PC1 against PC2 (Fig. 1) displays the distribution of compounds over the first two principal components space.

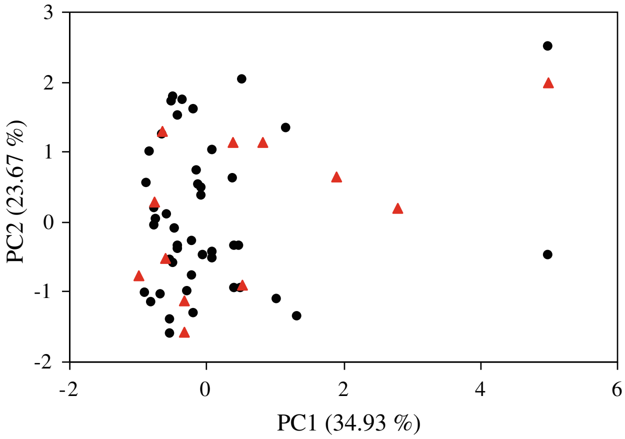


Fig. 1 Principal-components analysis of the training and test sets

According the results of PCA, all the data were divided into a training set of 44 compounds to develop the models and a test set of 11 compounds to evaluate the models based on two rules:

1 the range of the activity values of both the training set and the test set should be covered from the lowest to the highest;
2 the points corresponding to the training set in the PCA plot should not be out of the main clusters.

The two sets are listed in Table 1.

After analyzing splitting of the data set into the training and test sets, the next step was to select the main factors which were most important for the antibacterial activity of pleuromutilin derivatives. After descriptor calculation, both stepwise and genetic algorithms were performed to select descriptors correlated with the activity based on the training set samples. First, MLR analysis with stepwise selection and variables elimination was used to model the structure–activity relationships with a different set of descriptors. The stepwise MLR analysis led to the derivation of one model with six variables. It is described by the equation:

$$\begin{aligned} ED_{50} = {} & 67.634(\pm 11.809) - 568.889(\pm 123.974)\mathrm{X4A} \\ & + 4.196(\pm 2.523)\mathrm{GGI10} - 6.884(\pm 2.622)\mathrm{GATS5e} \\ & - 2.799(\pm 1.133)\mathrm{Mor30v} + 104.582(\pm 57.281)\mathrm{R3p}^{+} \\ & - 0.126(\pm 0.047)\mathrm{H-053} \end{aligned} \tag{1}$$

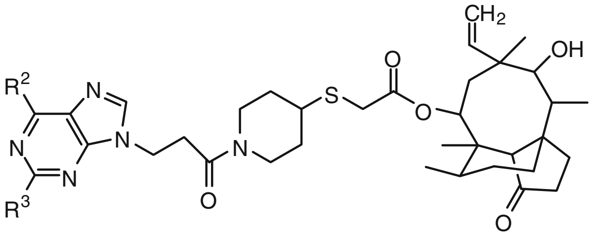
The model was then used to predict ED_{50} values for the compounds in the test set. The prediction results are given in Tables 1 and 2. The correlation coefficient, R^2, obtained was 0.793 for the training set and 0.723 for the test set. From Table 2 it can be seen that the SW–MLR model was not sufficiently accurate. Therefore, we applied genetic algorithms as the variable selection procedure to select only the best combinations of those most relevant to obtaining models with the highest predictive power by using the training set.

Results of the GA–MLR method

To select the optimum number of descriptors, the effects of the number of descriptors were investigated for one to eight descriptors. The effects of the number of descriptors on the coefficients of determination (R^2), the adjusted R^2 (R^2_{adj}) [11], and the coefficient of leave-one-out (LOO) cross-validation (Q^2) for the training set are shown in Fig. 2.

As can be seen, the models with seven and eight descriptors did not improve significantly the statistics of the model, thus it was determined that the optimum subset size had been achieved with a maximum of six descriptors. The six most significant descriptors according to the GA–MLR

Table 1 Experimental and predicted antibacterial activity (ED_{50}) of pleuromutilin derivatives by SW–MLR and GA–MLR models



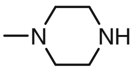
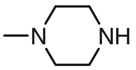
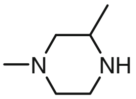
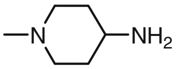
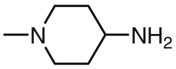
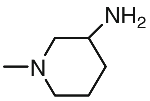
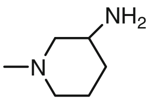
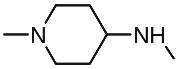
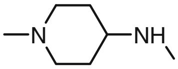
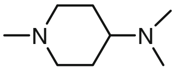
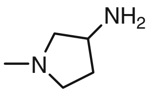
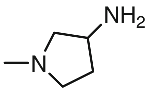
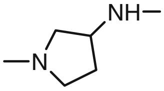
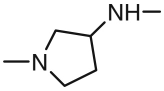
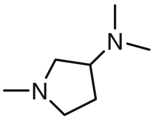
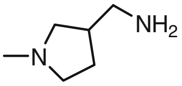
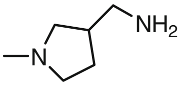
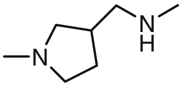
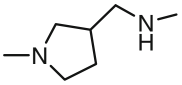
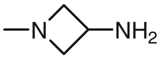
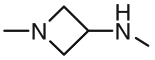
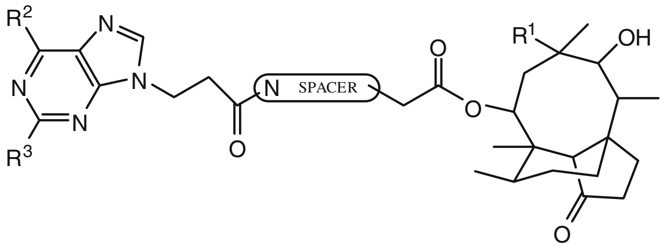
Number	R^2	R^3	ED_{50}	SW-MLR	GA-MLR
1	H	NH_2	2.89	2.64	2.57
2		NH_2	1.86	2.46	2.23
3		H	2.94	2.34	2.33
4		NH_2	2.21	2.19	2.42
5		NH_2	1.86	2.17	1.97
6		H	1.50	1.58	1.47
7		NH_2	2.21	2.32	2.35
8		H	2.21	2.14	2.33
9		NH_2	1.41	1.77	1.68
10		H	2.21	1.15	1.18
11		NH_2	1.72	1.99	2.18
12		NH_2	3.05	2.25	2.20
13		H	1.47	1.58	1.61

Table 1 continued

Number	R^2	R^3	ED_{50}	SW-MLR	GA-MLR
14		NH_2	1.72	2.31	2.26
15		H	1.61	1.64	1.63
16		NH_2	3.00	2.44	2.67
17		NH_2	2.84	2.24	1.90
18		H	0.59	1.43	1.12
19		NH_2	1.01	1.50	1.49
20		H	0.76	1.26	1.44
21		NH_2	3.04	3.31	3.38
22		NH_2	3.97	3.40	3.67



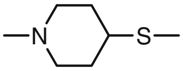
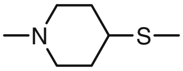
Number	R^1		ED_{50}	SW-MLR	GA-MLR
23A	Vinyl		1.86	2.36	1.89
24B	Vinyl		1.47	1.44	1.61

Table 1 continued

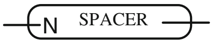
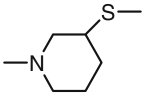
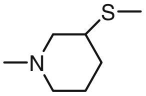
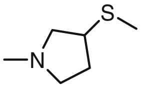
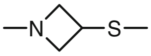
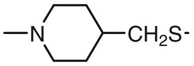
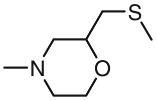
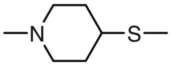
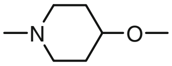
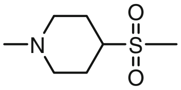
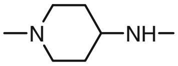
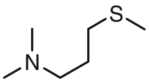
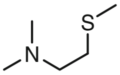
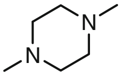
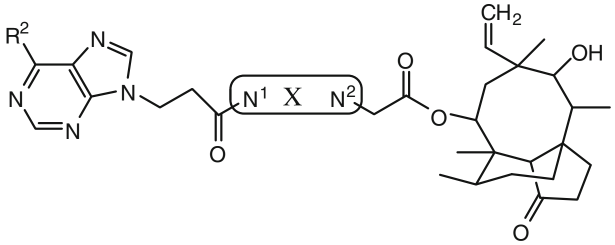
Number	R^1		*ED*$_{50}$	SW-MLR	GA-MLR
25A	Vinyl		3.13	3.36	2.90
26B	Vinyl		2.02	2.02	2.13
27A[a]	Vinyl		2.61	2.54	2.38
28A[a]	Vinyl		4.07	4.62	3.50
29A	Vinyl		2.77	2.43	2.01
30A[a]	Vinyl		4.81	4.31	5.28
31A	Ethyl		1.66	2.35	1.88
32B	Ethyl		2.04	1.64	1.97
33B	Ethyl		1.79	1.62	1.63
34B	Vinyl		1.75	1.40	1.76
35B	Vinyl		0.86	1.43	0.77
36B	Vinyl		1.76	1.98	1.49
37B	Vinyl		0.80	0.80	1.48

Table 1 continued



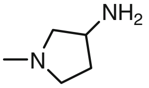
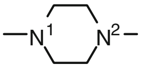
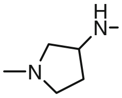
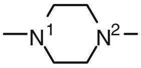
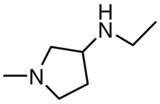
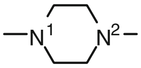
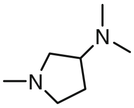
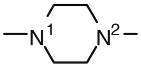
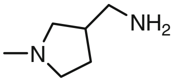
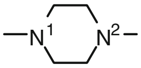
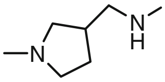
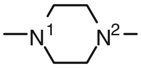
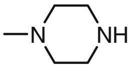
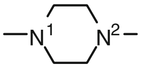
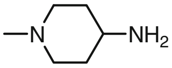
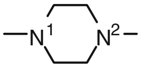
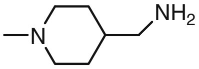
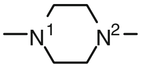
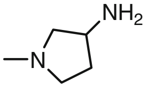
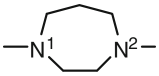
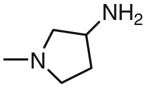
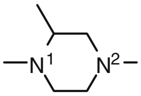
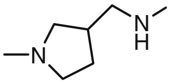
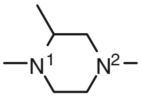
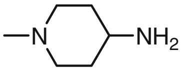
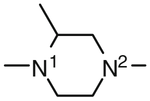
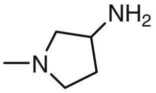
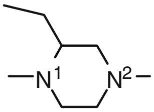
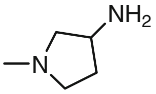
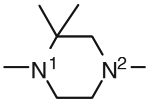
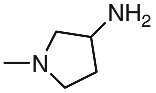
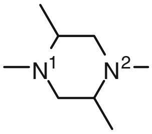
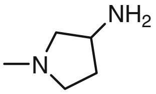
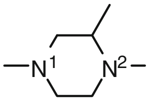
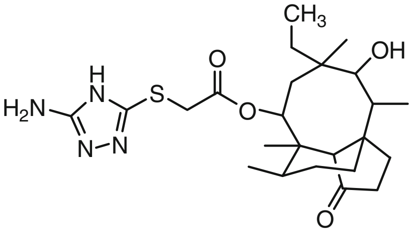
Number	R^2	X	ED_{50}	SW-MLR	GA-MLR
38[a]			0.80	1.38	1.47
39			1.30	1.44	1.32
40			2.21	1.21	1.80
41			2.94	1.72	2.18
42			1.21	1.49	1.08
43			1.10	1.21	1.26
44			1.65	2.04	1.94
45			0.97	1.33	1.25
46			1.51	1.23	1.66
47[a]			2.51	2.60	2.50
48			1.39	1.68	1.94
49[a]			1.20	1.60	1.87

Table 1 continued

Number	R^2	X	ED_{50}	SW-MLR	GA-MLR
50[a]			1.66	2.04	1.76
51[a]			2.95	1.80	2.29
52[a]			2.32	1.30	1.88
53[a]			3.13	2.80	3.49
54[a]			1.42	2.12	2.28
55			6.88	6.85	7.14

[a] Test set

A: R^2 = 1-piperazinyl, $R^3 = NH_2$

B: R^2 = 3-amino-1-pyrrolidinyl, $R^3 = H$

Table 2 Statistical results of different QSAR models

	Training set				Test set			
	R^2	Q^2_{LOO}	$F^a_{6,37}$	RMSE	R^2	Q^2_{LGO}	$F_{6,4}$	RMSE
SW–MLR	0.793	0.722	23.689	0.480	0.723	0.722	1.50	0.617
GA–MLR	0.839	0.760	32.113	0.423	0.807	0.771	2.702	0.523

[a] *F* is the variance ratio of calculated and observed values with two degrees of freedom

algorithm are: X4A, MATS5e, GATS7v, Mor12u, Mor17u, and H-053.

The chemical meaning and the mean effect (MF) of these descriptors are shown in Table 3. Also, multi-collinearity between these six descriptors was detected by calculating their variation inflation factors (VIF); these are shown in Table 3.

As can be seen from this table, most variables have VIF values less than 5, indicating that the model obtained has obvious statistical significance. With the selected

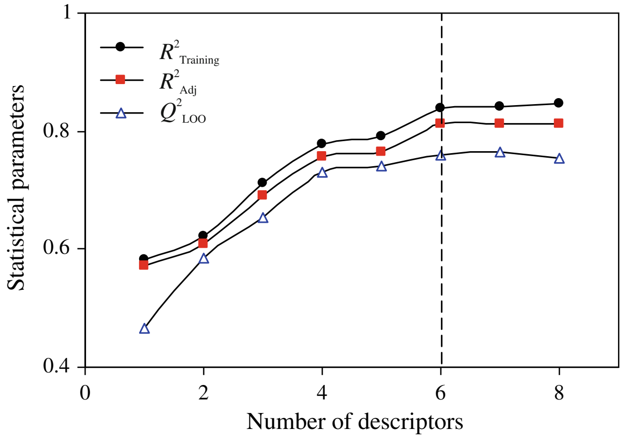


Fig. 2 Effects of the number of descriptors on the statistical data

descriptors, we built a linear model using the training set data, and the following equation was obtained:

$$\begin{aligned} ED_{50} = {} & 69.942(\pm 10.733) - 541.454(\pm 104.900)\mathrm{X4A} \\ & + 28.518(\pm 4.102)\mathrm{MATS5e} \\ & - 14.823(\pm 3.169)\mathrm{GATS7v} - 0.432(\pm 0.160)\mathrm{Mor12u} \\ & - 0.519(\pm 0.161)\mathrm{Mor17u} - 0.181(\pm 0.032)\mathrm{H-053} \end{aligned} \tag{2}$$

$$\begin{aligned} & N_{\mathrm{train}} = 44, R^2_{\mathrm{train}} = 0.839, F = 32.113, \\ & \mathrm{RMSE}_{\mathrm{train}} = 0.423, Q^2_{\mathrm{LOO}} = 0.760, \\ & Q^2_{\mathrm{LGO}} = 0.771, R^2_{\mathrm{test}} = 0.807, \mathrm{RMSE}_{\mathrm{test}} = 0.523 \end{aligned}$$

In this equation, N is the number of compounds, R^2 is the squared correlation coefficient, $Q^2_{\mathrm{LOO}}, Q^2_{\mathrm{LGO}}$ are the squared cross-validation coefficients for LOO and leave-group-out (LGO) respectively, RMSE is the root mean square errors (RMSEs), and F is the Fisher F statistic. The figures in parentheses are the standard deviations.

The built model was then used to predict the test set data. The prediction results are given in Tables 1 and 2. As can be seen from Table 1 the predicted values of vector ED_{50} are in good agreement with the experimental values. The values of ED_{50} predicted for the compounds in the training and test sets by use of Eq. 2 have been plotted versus the experimental values in Fig. 3.

The real usefulness of QSAR models is not just their ability to reproduce known data, verified by their fitting power (R^2)—it is mainly their possible predictive application. For this reason the model obtained was validated using LOO and LGO cross-validation processes. For LOO cross-validation, a data point is removed from the set, and the model is recalculated. The predicted activity for that point is then compared with its actual value. This is repeated until each data point has been omitted once. For LGO, 20% of the data points are removed from the dataset and the model was refitted; the predicted values for those points were then compared with the experimental values. Again, this is repeated until each data point has been omitted once. Cross-validation results are shown in Table 2. The cross-validated correlation coefficient (Q^2) is 0.771 for LGO and 0.760 for LOO. This confirms that the regression model obtained has good internal and external predictive power.

Also, in order to assess the robustness of the model, the Y-randomization test was applied in this contribution [7]. The dependent variable vector (ED_{50}) is randomly shuffled and a new QSAR model is developed using the original independent variable matrix. The new QSAR models (after several repetitions) are expected to have low R^2 and Q^2_{LOO} values (Table 4). If the opposite happens then an acceptable QSAR model cannot be obtained for the specific modeling method and data.

The applicability domain of this model was evaluated by leverage analysis expressed as a Williams plot (Fig. 4), in which the standardized residuals and the leverage values (h) were plotted. From this figure it is obvious that there are only three chemicals (52 in the training set and 55 and 30 in the test set) having leverage higher than the warning h^* value of 0.477, thus they can be regarded as structural outliers. Fortunately, in this case the data predicted by the model are good for compounds 52 and 55, thus they are “good leverage” chemicals. The analysis of the applicability domain highlighted the presence of a chemical outlier (compound 30) with standard residuals $>3\delta$. For this

Table 3 The linear model based on the six parameters selected by genetic algorithm method

Descriptor	Chemical meaning	Coefficient	Mean effect	VIF
Constant	Intercept	65.610	–	–
X4A	Average connectivity index chi-4	−502.590	0.804	1.706
MATS5e	Moran autocorrelation—lag 5/weighted by atomic Sanderson electronegativities	30.891	0.012	1.637
GATS7v	Geary autocorrelation—lag 7/weighted by atomic van der Waals volumes	−14.241	0.219	1.136
Mor12u	3D-MoRSE—signal 12/unweighted	−0.346	−0.015	1.349
Mor17u	3D-MoRSE—signal 17/unweighted	−0.561	−0.031	1.058
H-053	H attached to C0(sp3) with 2X attached to next C	−0.182	0.012	1.413

VIF variation inflation factors

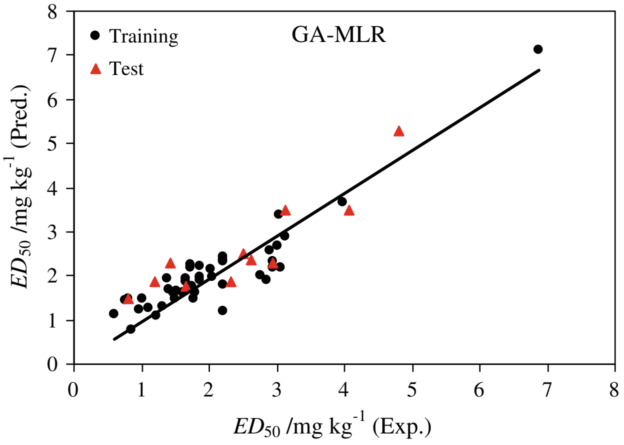


Fig. 3 Predicted versus experimental antibacterial activity (ED_{50}) by GA–MLR

Table 4 R^2_{train} and Q^2_{LOO} values after several *Y*-randomization tests

Iteration	R^2_{train}	Q^2_{LOO}
1	0.053	0.123
2	0.202	0.004
3	0.061	0.084
4	0.085	0.001
5	0.090	0.001
6	0.146	0.002
7	0.154	0.027
8	0.238	0.037
9	0.078	0.043
10	0.120	0.001

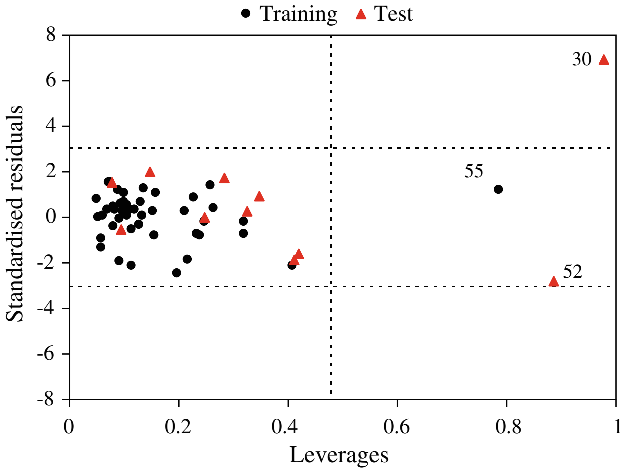


Fig. 4 Williams plot of the GA–MLR model. The training and test set samples are labeled differently. The *dashed lines* are the 3δ limit and the warning value of hat ($h^* = 0.477$). Compounds with high *h* values are numbered as in Table 1

compound, the experimental $ED_{50} = 4.81$, and the model prediction was 5.28.

Table 2 presents the statistical data for the results obtained from the two studied models for the same set of compounds. The RMSE of the GA–MLR method for the training and test data sets were lower than those of the model proposed in the SW–MLR method. The correlation coefficient (R^2) given by GA–MLR was higher than that of the SW–MLR. The results of the *F*-test were obtained and also are shown in Table 2. From this table it can be seen that the GA–MLR model gives higher *F* values, so this model gives the most satisfactory results, compared with the results obtained from the SW–MLR method. Consequently, this GA–MLR approach currently constitutes the most accurate method for prediction of the antibacterial activity of pleuromutilin derivatives.

Molecular descriptor interpretations

Table 3 lists the molecular descriptors selected by the genetic algorithm as the most relevant in their combination for the prediction of antibacterial activity. The average connectivity index (X4A) is a topological descriptor calculated from the vertex degree of the atoms in the H-depleted molecular graph. As is apparent from Table 3, the X4A mean effect has a positive sign, illustrating a greater mean effect value than that of the other descriptors. Therefore, this descriptor had a significant effect on the antibacterial activity of pleuromutilin derivatives. The positive sign suggests that the ED_{50} value is directly related to this descriptor. The two 2D-autocorrelation descriptors (MATS5e of Moran [12] and GATS7v of Geary [13]) give information on the distribution of the atomic properties along the topological structure. The weight properties are here an electronic property (atomic electronegativity) that highlights the relevance of the polarity properties for antibacterial activity, and atomic volume, encoding information on molecular dimension. Table 3 shows that MATS5e and GATS7v mean effects have positive signs, which indicate that antibacterial activity is directly related to polarity and volume of the molecules. Therefore, increasing the polarity and volume of the molecule leads to an increase in its antibacterial activity (ED_{50} value). The fourth and fifth descriptors are Mor12u and Mor17u, which belong to the 3D MoRSE descriptors. These descriptors were proposed as signal 12 and 17 unweighted. 3D-MoRSE (3D-molecule representation of structures based on electron diffraction) descriptors are based on the idea of obtaining information from the 3D atomic coordinates by the transformation used in electron diffraction studies for preparing theoretical scattering curves. It is worth noting that they reflect the three-dimensional arrangement of the atoms of a molecule and nothing about chemical bonds. In general, the 3D descriptors can add valuable information to the models. Mor12u and Mor17u display a negative sign, which indicates that the ED_{50} is inversely related to these descriptors. H-053 (H attached to C0(sp3) with 2X attached to next C) is the last descriptor appearing in the model. It is one of the atom-centered fragment descriptors that describes each atom by its own atom type and the bond types and atom types of its first neighbors. This descriptor represents the first neighbor (hydrogen) of carbon atoms. As can be seen from Table 3 H-053 mean effect has negative sign, which indicates that with decreasing the number of carbon atoms neighboring hydrogen (C0(sp3)) the ED_{50} values increase, which indicates that structural features which are related to antibacterial activity should have low number of hydrogen atoms as neighbors of carbon atoms (C0(sp3)).

Conclusions

QSAR analysis was performed on antibacterial activity of pleuromutilin derivatives by use of the MLR procedure. For each molecule 1481 theoretically derived descriptors were calculated. The best set of calculated descriptors was selected with the stepwise and genetic algorithms. The results obtained by GA–MLR were compared with those obtained by SW–MLR which confirmed the superiority of the GA–MLR model as a more powerful method for predicting antibacterial activity. A suitable model with high statistical quality and low prediction errors was eventually derived. The proposed model could identify and provide some insight into which structural features are related to the antibacterial activity of pleuromutilin derivatives.

Materials and methods

Data set

The 55 novel pleuromutilin derivatives studied and their corresponding antibacterial activities were collected from Refs. [3–5]. The activities of these compounds were expressed by ED_{50} (half maximum effective dose) that are listed in Table 1.

Data splitting

The original data set was split into a training set (44 compounds), used for establishing the QSAR model, and a test set (11 compounds) for external validation. The diversity of the training set and test set was analyzed by use of principal-components analysis (PCA) [14, 15].

Descriptors calculation

The 2D structures of the compounds were pre-optimized using the MM+ molecular mechanics force field in HyperChem [16]. The final geometries of the minimum energy conformations were obtained by more precise optimization with the semi-empirical AM1 method. Then 1481 theoretical molecular descriptors were calculated by use of the DRAGON program [17], including:

1 0D-constitutional descriptors;
2 1D-functional groups, atom centered fragments;
3 2D-topological descriptors, walk and path counts, connectivity index, information index, various autocorrelations from the molecular graph, edge adjacency indices, descriptors of Burden eigenvalues, topological charge indices, eigenvalue-based indices;
4 3D-Randic molecular profiles, geometrical descriptors, weighted holistic invariant molecular descriptors (WHIMs), geometry, topology and atom-weights assemblY (GETAWAY) descriptors;
5 charge descriptors; and
6 molecular properties (calculated from models, together with some empirical descriptors).

The list and meaning of the molecular descriptors can be obtained from the DRAGON package, and the calculation procedure is explained in detail in the Handbook of Molecular Descriptors [18]. The theoretical descriptors were reduced by the following procedure:

1 descriptors that are constant were eliminated;
2 in addition, to reduce the redundancy existing in the descriptors, the correlation of descriptors with each other and with ED_{50} of the molecules were examined, and collinear descriptors ($R > 0.9$) were detected. Among the collinear descriptors, the one with the highest correlation with ED_{50} was retained, and the others were removed from the data matrix.

Genetic algorithm (GA)

Genetic algorithms (GAs) are governed by biological evolution rules [19]. They are stochastic optimization methods that have been inspired by evolutionary principles. The distinctive aspect of a GA is that it investigates many possible solutions simultaneously, each of which explores different regions in parameter space [20]. The first step is to create a population of N individuals. Each individual encodes the same number of randomly chosen descriptors. The fitness of each individual in this generation is determined. In the second step, a fraction of children of the next generation is produced by crossover (crossover children) and the rest by mutation (mutation children) from the parents on the basis of their scaled fitness scores. The new offspring contains characteristics from two or one of its parents. The GA method is especially useful when finding the global optimum is harmed by the presence of many local optima in a complex response hypersurface. GA succeeds whilst the presence of local optima makes direct optimization methods unreliable and the exhaustive search is impossible for high-dimensionality problems. The GA program was written in Matlab 6.5 [21].

Model validation and applicability domain

Before a QSAR model is used to predict the activity of new compounds, it should be validated both internally and externally to ensure that the built model is robust, stable, and predictive. In this work, several statistics terms, for example correlation coefficient (R^2), LOO cross-validated Q^2, and RMSE were used to assess the internal predictive ability of the proposed models. Y-scrambling techniques were also used to exclude the possibility of chance correlation and to check for reliability and robustness by permutation testing. Multi-collinearity between the selected descriptors was detected by calculating their VIF, which can be calculated as follows:

$$\mathrm{VIF} = \frac{1}{1 - r^2} \qquad (3)$$

where r is the correlation coefficient of multiple regression between one variable and the others in the model. If VIF equals 1.0, no intercorrelation exists for each variable; if VIF falls into the range 1.0–5.0, the related model is acceptable; and if VIF is larger than 10.0, the related model is unstable and rechecking is necessary [22]. To examine the relative importance and the contribution of each descriptor to the model, the value of the mean effect (MF) was calculated for each descriptor by use of the equation:

$$\mathrm{MF}_j = \frac{\beta_j \sum_{i=1}^{i=n} d_{ij}}{\sum_j^m \beta_j \sum_i^n d_{ij}} \qquad (4)$$

MF_j represents the mean effect for the considered descriptor j, β_j is the coefficient of the descriptor j, d_{ij} stands for the value of the target descriptors for each molecule and, eventually, m is the descriptor number in the model. The MF value indicates the relative importance of a descriptor, compared with the other descriptors in the model. Its sign exhibits the direction of variation in the values of the activities as a result of the increase (or reduction) of this descriptor value.

The Williams plot, the plot of the standardized residuals versus the leverage, was used to visualize the applicability domain [23]. Leverage indicates a compound's distance from the centroid of X. The leverage of a compound in the original variable space is defined as [24]:

$$h_i = x_i^{\mathrm{T}} \left(X^{\mathrm{T}} X\right)^{-1} x_i \qquad (5)$$

where x_i is the descriptor vector of the considered compound and X is the descriptor matrix derived from the training set descriptor values. The warning leverage (h^*) is defined as [25]:

$$h^* = 3(p+1)/n \qquad (6)$$

where n is the number of training compounds and p is the number of predictor variables. A compound with $h_i > h^*$ seriously affects the regression performance, but it does not seem to be an outlier because its standardized residual may be small, even though it has been excluded from the applicability domain. Moreover, a value of 3 for standardized residual is commonly used as a cut-off value for accepting predictions, because points that lie ±3 standardized residuals from the mean cover 99% of normally distributed data [26]. Thus the leverage and the standardized residual were combined for characterization of the applicability domain.

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