Probabilistic Neural Network Model for the In Silico Evaluation of Anti-HIV Activity and Mechanism of Action

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A theoretical model has been developed that discriminates between active and nonactive drugs against HIV-1 with four different mechanisms of action for the active drugs. The model was built up using a probabilistic neural network (PNN) algorithm and a database of 2720 compounds. The model showed an overall accuracy of 97.34% in the training series, 85.12% in the selection series, and 84.78% in an external prediction series. The model not only correctly classified a very heterogeneous series of organic compounds but also discriminated between very similar active/nonactive chemicals that belong to the same family of compounds. More specifically, the model recognized 96.02% of nonactive compounds, 94.24% of active compounds that inhibited reverse transcriptase, 97.24% of protease inhibitors, 97.14% of virus uncoating inhibitors, and 90.32% of integrase inhibitors. The results indicate that this approach may represent a powerful tool for modeling large databases in QSAR with applications in medicinal chemistry.

Introduction

Acquired immunodeficiency syndrome (AIDS) is currently a very serious health problem despite the efforts of researchers worldwide to unravel the mode of action of the virus and develop efficient treatments. HIV, the causative agent of AIDS, has been identified as a retrovirus from the *Lentiviridae* family.[1,2] There are currently different therapeutic targets that can be used as candidates for drug lead development in this area. The new drug candidates are intended to act mainly as target inhibitors, and the most widely studied compounds are reverse transcriptase inhibitors (T), protease inhibitors (P), virus uncoating inhibitors (U), and integrase inhibitors (I). In particular, the compounds that act by mechanisms T and P have been studied in more depth and can generally be distinguished as nucleoside RT inhibitors and nonnucleoside RT inhibitors for group T and peptidic versus nonpeptidic inhibitors for group P. The number of compounds that has been assayed is so large that different authors have introduced computer-aided drug design techniques. In general, there are two important classes of computer-aided drug design techniques based either on drug−target interactions driven by molecular forces (Docking)[3] or quantitative structure−activity relationships (QSAR) using molecular descriptors.[4,5]

The main steps involved in developing a QSAR model are (a) selection of the dataset, (b) calculation of molecular descriptors, (c) fitting the statistical model, and (d) validation of the model. Numerous different molecular descriptors have been reported to encode chemical structure in QSAR studies. Among the most relevant indices or molecular descriptors that can be used in step b are the 3D, 2D, and 1D descriptors, and these include the so-called topological[6,7] and quantum-chemistry descriptors.[8] Furthermore, there are multiple chemometric approaches that can in principle be selected for step c.[9] Multiple linear regression (MLR), linear discriminant analysis (LDA), partial least-squares (PLS), and different kinds of artificial neural networks (ANN) can be used to relate molecular structure (represented by molecular descriptors) with biological properties. The ANNs are particularly useful in QSAR studies in which the linear models fit poorly due to high data complexity.

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There are different kinds of ANNs, and these include multilayer perceptron (MLP), radial basis functions (RBF), and PNN—this ANN is a variant of RBF systems.[10,11] In particular, PNN is a type of neural network that uses a kernel-based approximation to form an estimate of the probability density functions of classes in a classification problem. PNNs have different advantages with respect to other ANN, and these include short training times, the possibility of rapid processing of large databases, and unnecessary network architecture optimization. The latter factor is predetermined by the number of input cases (molecular descriptors), the number of hidden units (one per compound of the training), and the number of output categories (number of mechanisms of action plus one to account for the nonactive drugs group). PNNs are multiple-category classifiers, i.e., one can model the biological activity of different compounds by regarding different targets using a single PNN. On the basis of the considerations outlined above, 1D and 2D descriptors and a PNN algorithm are used here to achieve the objective of the present work: the introduction for the first time of a general purpose model that discriminates between structurally heterogeneous nonactive and active anti-HIV-1 compounds with different mechanisms of action. This model could prove to be of major interest in the future in the mining of large databases to find novel molecular scaffolds with potential anti-HIV-1 activity and also as an alternative to select the drug target for drug−target interaction (docking) studies.

Methodology

Database. A large series of 2720 compounds was collected from the literature. Different types of anti-HIV-1 compounds acting by different mechanisms of action were included: group T compounds (HEPT, TIBO, TSAO, nevirapine, pyridinone, nucleosides, and other derivatives), group P compounds (cyclic urea, peptidic, cyclopyranones, and penicillin derivatives), group U compounds (bis-tetraazamacrocyclic derivatives), and group I compounds (tyrphostins, coumarins, aromatic sulfonamides, chicoric acids, tetracyclines, curcumins, and others). In addition, group N is composed of both nonactive compounds that represent of all of the classes mentioned above as well as very heterogeneous compounds from different families.

The original database of 2720 compounds was divided into three different series: a training series of 2369 compounds (774 anti-

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Table 1. Molecular Descriptors Used in the Classification Model and Their Definitions

descriptors	definition
MR[a]	Ghose−Crippen molar refractivity
PSA[a]	fragment-based polar surface area
MLogP[a]	Moriguchi octanol−water partition coeff. (logP)
X3[b]	connectivity index chi-3
X3sol[b]	solvation connectivity index chi-3
S0K[b]	Kier symmetry index
SEIge[b]	eigenvalue sum from electronegativity weighted distance matrix
VEA1[b]	eigenvector coefficient sum from adjacency matrix

[a] Properties descriptors. [b] Topological descriptors.

HIV-1 and 1595 nonanti-HIV-1 compounds), a selection or supervised cross-validation series of 121 compounds (52 anti-HIV-1 and 69 nonanti-HIV-1 compounds), and an external prediction series of 230 compounds (83 anti-HIV-1 and 147 nonanti-HIV-1 compounds). The training series was used to train the PNN, the selection series was used to supervise the function of the PNN during training, and the external prediction series was not used to train the PNN but was used to validate the predictive power of the model. The selection series helped to train the network and determined the training stop point in order to avoid over-fitting problems. Different EC_{50} and/or IC_{50} threshold limits were used to assign a compound as active in a specific group: $EC_{50} \leq 0.50$ μM is the limit for group T, $IC_{50} \leq 0.0150$ μM in an HIV−PR binding affinity assay was the limit for group P, $EC_{50} \leq 0.50$ μM is the limit for group U, and 3′-processing $IC_{50} \leq 3.0$ μM was the limit to include a compound as active in group I. Otherwise, the compounds were included in group N, i.e., nonanti-HIV-1 compounds. All compounds in group T from the same structural family were evaluated in the same cellular line, which is specified in the tables in the Supporting Information. The selection of different threshold limits for each group was governed by two factors: (a) differentiation between active and nonactive compounds in terms of reasonable activity values and (b) the design of series of compounds with a proportional number of active/nonactive compounds in these series.

Computational Software. All of the molecular structures were drawn with the software HyperChem, and the structural information was saved as output coordinate (.hin) files.[12] These files were subsequently used as inputs for software used in the calculation of the molecular descriptors.

Molecular Descriptors. The software Dragon[13] was used to calculate 1D (functional groups, atom-centered fragments, empirical descriptors, and properties) and 2D molecular descriptors (topological descriptors, molecular walk counts, BCUT descriptors, Galvez topological charge indices, and 2D autocorrelations) based on the .hin files generated with HyperChem. Optimization of the geometry was not necessary due to the nature of these molecular descriptors. For the sake of simplicity, only the descriptors selected by the PNN as statistically significant are depicted in Table 1.[14]

Feature Selection. A genetic algorithm was used to select the molecular descriptors with the largest influence on the biological activity.[15]

PNN Modeling. The neural network was trained using the algorithm previously implemented in the STATISTICA software package.[15] The kernel-based approach used here for the probability density function approximation is very similar to radial basis function networks (RBF) and motivates the probabilistic neural network (PNN) and generalized regression neural network (GRNN), both of which were devised by Specht.[10,16] PNNs are designed for classification tasks and GRNNs for regression.

There are four layers in the PNN: input, hidden, summation, and output layers. The hidden or radial units are copied directly from the training data (one per case). Each unit models a Gaussian function centered on the training case. There is one output unit per class. Each unit is connected to all the radial units belonging to its class, with zero connections from all other radial units. Hence, the output units simply add up the responses of the units belonging to their own class. The outputs are each proportional to the kernel-based estimates of the probability density function for the various classes, and normalization of these to sum to 1.0 produces estimates of class probability.

PNN uses a probability density function for each category (drugs group), and this has the following general formula:

$$f_A(x) = \frac{1}{m(2\Pi)^{p/2}\sigma^p} \sum_{i=1}^{m} \exp\left[-\frac{(x - x_{Ai})^T (x - x_{Ai})}{2\sigma^2} \right]$$

where $f_A(x)$ represents the probability density function for category A with a vector random variable x, m is the number of training patterns, p is the number of independent features (descriptors), x_{Ai} is ith training pattern from category A and σ is the width of the Gaussian-shaped kernels. The topology of the neural network developed in this paper have 8 inputs, 2369 radial units, 5 summation units, and 1 output.

Results and Discussion

The model developed in this study has an overall good classification percentage of 97.34% in the training series and 85.12% in the selection series (see Table A in the Supporting Information). A total of 84.78% of the compounds in the external prediction series are correctly identified by the classification model (see Table B in the Supporting Information). The classification function for the prediction group shows excellent results, and these confirm the quality of the trained network. Analysis of the classification by category shows that the model correctly evaluates 94.24% for (T), 97.24% for (P), 97.14% for (U), 90.32% for (I), and 96.02% for (N). Families of compounds were introduced into the database that have anti-HIV activity along with others that not present this activity. Within the different families that do show anti-HIV activity there are both active and inactive compounds. In this way, information is introduced into the network with the aim of making the model capable of optimizing the activity of a given family, and at the same time, the model could have the predictive capacity to find new lead compounds—although it should be noted that such predictions are not straightforward. The classification function evaluated the three series of compounds with a high level of certainty and is capable of discriminating between compounds from the same family even when they are structurally very similar. This ability gives the model the potential to optimize the anti-HIV activity within a given family of compounds. The percentages of correct classifications for the different families of compounds in the external prediction series are shown in Figure 1.

The model classifies compounds into five categories according to the therapeutic target, which enables the model to establish not only the activity but also the mechanism of action of the compound. The five categories are the following:

Reverse transcriptase inhibitors (T). The compounds introduced include a series of HEPT derivatives, TSAO analogues, TIBO derivatives, nevirapine derivatives, pyridinone derivatives, α-APA derivatives, thiadiazole derivatives, colchicine derivatives, benzophenones, quinolines, and nucleosides. The neural network gives very good classification percentages for each of these families of compounds. The correct classification percentages in the training and selection series were 96.43% and 89.66%, respectively. The external prediction series (see Table B in the Supporting Information) is evaluated in this category with a level of success of 73.0%. The percentages of good classification for the different families in category (T) are shown in the tables in the Supporting Information. The network retains a level of symmetry in that virtually all of the groups are evaluated well.

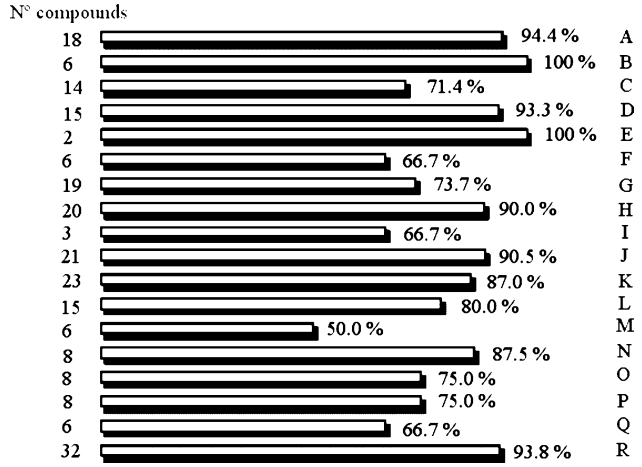

Figure 1. Graph depicting the percentages of good classification for the external predicting series. Reverse transcriptase inhibitors (A = HEPTs; B = TSAOs; C = nevirapine analogues; D = pyridinones; E = thiadiazoles, benzophenones; F = colchicine, PETTs; G = TIBOs; H = nucleosides; I = thiocarboxanilide analogues). Protease inhibitors (J = cyclic ureas; K = pyranones; L = peptides; M = penicillin analogues). Uncoating inhibitors (N = bis-tetraazamacrocycles). Integrase inhibitors (O = coumarins, styrylquinolines, curcumins; P = salicylhydrazines, cinnamoyl-based, sulfonamides, chicoric acids; Q = thiazolothiazepines, depsides, tyrphostins, arylamides). Nonactive families (R).

Protease inhibitors (P). This group consists of cyclic urea derivatives, peptide derivatives, pyranones, and penicillin derivatives. The training and selection series give success levels of 98.24% and 100%, respectively. The percentage of compounds evaluated correctly in the external prediction series is 86.67%, which clearly demonstrates the predictive power of the model. The four structural groups that make up this category are evaluated correctly in all three series.

Virus uncoating inhibitors (U). A series of bis-tetraazamacrocyclic compounds was introduced. The levels of correct classification in the training, selection, and prediction series were 100%, 100%, and 85.71%, respectively.

Integrase inhibitors (I). Tyrphostins, coumarins, aromatic sulfonamides, chicoric acids, tetracyclines, arylamides, thiazolothiazepines, curcumins, salicylhydrazines, styrylquinolines, and depsides were introduced. The percentages of correct classification were 95.56% in the training series and 75.0% in the selection series. The predictions series also showed a good level of compound classification (77.78%).

Inactive compounds (N). The inactive compounds were correctly evaluated in all three series: 97.43% in the training series, 81.16% in the selection series, and 87.76% in the external prediction series. The neural network proved to be capable of reliably predicting both the inactive compounds that belong to active families as well as those from inactive families.

To demonstrate the potential of our model to identify lead compounds, a new calculation was undertaken in which the PETT derivatives (Table T15 of the Supporting Information) were removed from the training and selection series and introduced into the prediction series. This approach ensures that the model does not have any information on this active family of compounds. The model recognized 11 of the active compounds from this structural family and also correctly classified them into category T. This demonstrates the predictive capability of our model both in terms of identifying lead compounds and to classify their mechanism of action.

There are various methods available to validate a given statistical model. The stability of the model was confirmed by exchanging a number of compounds from the training series with compounds chosen at random from the selection series. The percentages in the training, selection, and external prediction series remained unchanged, thus demonstrating the consistency of the network. The results were similar to those described above and can be seen in Table 2.

Table 2. Description of the Models Found with Different Selection Series

	% training	% selection	% prediction
model 1[a]	97.34	85.12	84.78
model 2	95.27	81.82	85.65
model 3	97.38	85.65	79.34
model 4	98.14	81.82	85.21
model 5	97.26	86.00	83.47

[a] Model described in this paper.

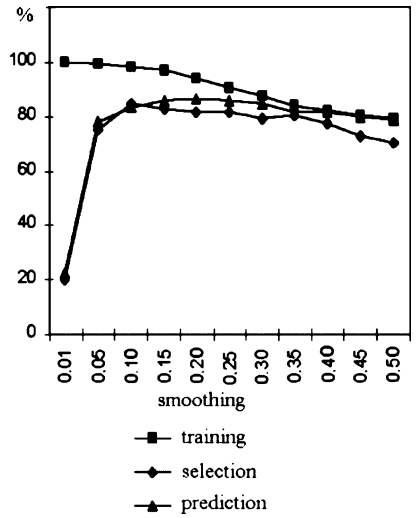

Figure 2. Effect of varying the smoothing value on the classification percentages in the training, selection, and prediction series.

The smoothing factor determines the widths of the Gaussian functions. If the smoothing value is high, then the error in selection is low and that in training is high. On the other hand, a low smoothing value favors training but the selection suffers. A stability study was carried out on the network by varying the smoothing values in the range 0.01−0.50 (see Figure 2). Values between 0.01 and 0.10 lead to significantly improved percentages of good classification in the selection and prediction series, while the level of success for the training series falls slightly. The optimum smoothing value is between 0.10 and 0.15 in that good levels of classification are obtained in all three series. Smoothing values above 0.15 cause a decrease in the classification percentages, and the model diverges from the optimum smoothing value.

Receiver Operating Characteristic (ROC) curves for the training, selection, and predicting series were also produced by varying the a priori probabilities from 0.9 to 0.1 using Origin software.[17] This type of curve differentiates between two categories, and for this reason, the active compounds with different mechanisms of action were combined into a single category. The area under the curve is 0.995 for the training series, 0.874 for the selection series, and 0.893 for the external prediction series. These results confirm that the developed model is not a random classifier on the basis that the area values are significantly higher than 0.5 (see Figure 3).

Finally, the results obtained with PNN were compared with those obtained using another type of neural network (multilayer perceptron, MLP) and by linear discriminant analysis (LDA). The percentages of good classification for the different catego-

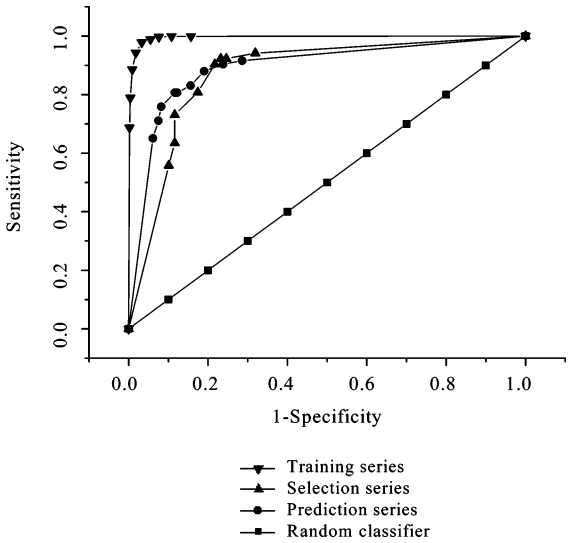

Figure 3. Receiver operating characteristic curve (ROC curve) for training, selection, and prediction series of active (T, P, U, I) and nonactive (N) compounds.

Table 3. Comparison of the Results Obtained for the Different Categories (N, T, P, U, I) Using Probabilistic Neural Network, Multilayer Perceptron, and Linear Discriminant Analysis

	Models				
	PNN[a]				
	N	T	P	U	I
% training	97.4	96.4	98.2	100	95.6
% selection	81.2	89.7	100	100	75.0
% prediction	87.8	73.0	86.7	85.7	77.8
	MLP[b]				
	N	T	P	U	I
% training	75.5	48.5	75.0	92.0	31.1
% selection	71.0	58.6	83.3	100	50.0
% prediction	72.7	56.7	80.0	85.7	44.4
	LDA[c]				
	N	T	P	U	I
% training	40.2	64.8	76.4	100	75.6
% selection	34.8	72.4	91.7	100	100
% prediction	40.8	64.9	80.0	85.7	77.8
	LDA[d]				
	N	T	P	U	I
% training	63.5	52.4	92.6	100	80.0
% selection	65.2	37.9	91.7	100	87.5
% prediction	65.3	64.9	100	100	77.8

[a] Model described in this paper. [b] Multilayer perceptron model with the same variables as the PNN. [c] Linear discriminant analysis with the same variables as the PNN. [d] The best linear discriminant analysis with eight variables from Dragon selected by the *stepwise* method.

ries are far higher in the case of PNN (see Table 3). In addition, this finding confirms that it was necessary to model this database using a technique that would allow us to establish a nonlinear model with the capability of learning (e.g., PNN).

Conclusion

The study described here concerned a very complex database and enabled the differentiation between compounds with anti-HIV activity and those without. The database contained both active and inactive compounds from families that inhibit the virus as well as a series of inactive compounds. The compounds had a wide range of different structures. It was not possible to develop a linear model, but it was found that nonlinear models established with techniques such as neural networks have a good predictive capacity. This finding is important when dealing with large databases that are difficult to adjust with linear models.

The use of PNN also led to a study aimed at predicting the mode of action of the different inhibitors—an approach that will aid the selection process for biological assays required to verify the activity of a given compound.

The approach described here could represent a powerful tool in the design of novel pharmacological agents and allow the analysis of large databases to identify new compounds with potent activity.

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Supporting Information Available: Tables A and B[18−80] listing the compounds that form the training, selection, and prediction series for the theoretical models. This material is available free of charge via the Internet at http://pubs.acs.org.

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