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DPPH• Free Radical Scavenging Activity of Coumarin Derivatives. *In silico* and *in vitro* Approach

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Abstract: The interest of coumarins as antioxidant agents has attracted much attention in recent years. A quantitative structure-activity relationship (QSAR) study of the DPPH• (*2,2-diphenyl-l-picrylhydrazyl*) radical scavenging ability of chemical compounds, based on the 0-3D DRAGON molecular descriptors and an artificial neural networks (ANN) technique was developed. The built mathematical model showed a correlation coefficient for the training set (R^2) = 0.71, an external correlation coefficient (Q^2_{ext}) = 0.65 and it was used to predict the antioxidant activity of 4-hydroxycoumarin derivatives. Besides, an experimental *in vitro* assay was developed for the reference compound of this group (4-hydroxycoumarin) and the results obtained confirmed the predictions made by the ANN.

Keywords: coumarin; artificial neural networks; antioxidant; QSAR; DPPH; free radical scavengers

1. Introduction

The development of antioxidant agents has attracted much attention in recent years, because oxidative damage is related to many pathological conditions [1]. Several coumarin derivatives have been studied for their biochemical and pharmacological profiles. Some studies suggest that these compounds may significantly affect the function of various mammalian cellular systems. Specifically their antioxidant effect has been explored, because the structural features of this group of compounds suggest that they can probably exhibit this pharmacologic property [2-4]. The antioxidant capacity can be experimentally measured by several *in vitro* assays. One of the best-known method is the one based on the capturing of the DPPH• radical [5,6].

Chemoinformatics tools have been used in the modeling of the antiradical activity, as well as others biological properties, given their advantages in saving time and resources [7,8]. Several statistical and machine learning methods have been widely used in the literature to build models for studying Quantitative Structure Activity Relationships (QSAR). The QSAR studies assess mathematical associations between structural features of the molecules and biological properties. For the last two decades, Artificial Neural Networks (ANN) have increasingly found applicability in QSAR studies, thanks to their ability to map non-linear relations between structural characteristics of chemical compounds and their chemical / biological behavior [9].

The *objective* of this study was to develop an ANN model in order to relate the chemical compounds' scavenging ability of the DPPH• radical with the corresponding structural features, also known as molecular descriptors (MDs). Then, an experiment to predict the antioxidant activity of a group of coumarin derivatives was performed. The coumarin-related compounds used as models in this study; were previously synthesized by the team of Molecular Chemistry at Cnam, Paris.

2. Results and Discussion

2.1. Modeling: The mathematical Multilayer Perceptron (MLP) neural model was constructed using DPPH• scavenging capacity of 1329 compounds reported in the literature. This model showed a correlation coefficient ($\boldsymbol{R^2}$) for the training set of 0.71. The predictive ability of the model was assessed using the external validation procedure, yielding a correlation coefficient ($\boldsymbol{Q^2_{ext}}$) of 0.65. Both values are above the limits established for model acceptance [10], and thus indicate the fitness and predictive power of the obtained ANN model.

2.2. Prediction: Recent advances in drug discovery have enabled a dramatic increase in the number of synthetic and naturally occurring molecules that are available for testing using *in vitro* assays as the scavenging ability of the DPPH• radical. Virtual screening allows for prior assessment of the potential bioactivity of chemical compounds, and thus providing key guidelines in posterior experimental work [11]. In this study, the MLP model obtained was used to predict DPPH• scavenging capacity of coumarin derivatives which were divided into 2 groups, following their structural analogy in function of the posterior analysis of their activity.

-*Group1*: cyclocoumarol analogous (**Cy-analogs**)

-*Group2*: warfarine analogous (**Wf-analogs**).

The results of the predictions for both groups are shown in **Table 1**. *Group1* (compounds **1-7**) and *Group2* (compounds **8-15**) have significantly different values of pIC_{50} as it can be noticed. **Cy-analogs** clearly seems to be less effective in

DPPH• radical capturing, because their values of pIC_{50} are highest (around 4). On the other hand, the pIC_{50} **Wf-analog** values are under 3.3. In the case of 4-hydroxycoumarin (number 15) the pIC_{50} value obtained was of 3.4. These results indicate the superior ability of the compounds *Group2* for scavenging the DPPH• radical. To justify the observed trends, a more detailed analysis of the structural characteristics is required. Firstly, the presence of a free hydroxyl group in **Wf-analogs** may probably favor the higher antioxidant activity of this group. In fact, several research studies have pointed hydroxyl groups as key for antiradical capacity and consequently, antioxidant activity [8, 12-15]. The hydroxyl group is present in the most frequently used reference compounds like: trolox, gallic acid or butylated hydroxytoluene (BHT).

2.3. In vitro Assay: The results obtained with *in silico* modeling were corroborated by an *in vitro* study of DPPH• scavenging capacity for the reference compound, 4-hydroxycoumarin. Selecting this compound was specifically based on the prediction results obtained because, according to the model, this molecule has an intermediate pIC_{50} value. The experimental result of the *in vitro* assay can provide a comparison criterion for evaluating the neural model and its predictions.

The experimental *in vitro* pIC_{50} value (2.7) obtained according to the method described below for 4-hydroxycoumarin is to be compared with the prediction value 3.4 given by the model. This result is positive, because the neural model as well as the *in vitro* assay are in the scale of high activity according to the statistical range established by the built Data and also compared to the value obtained experimentally for BHT (2.10) use as reference.

It may thus be suggested that the 4-hydroxycoumarin possesses significant radical scavenging ability, and therefore it can be considered as a candidate for antioxidant agent; although more analyses are necessary to ensure that.

The results obtained in the *in vitro* assay confirmed the predicting power of designed ANN model, and consequently its applicability in the search for new antioxidant compounds. An *in vitro* study of antioxidant activity of warfarine analogues is currently underway.

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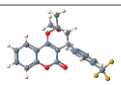
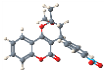
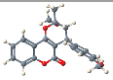
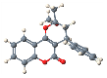
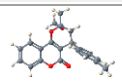
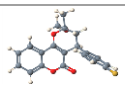
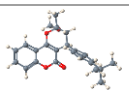
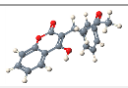
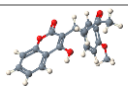
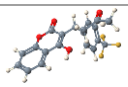
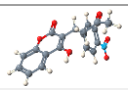
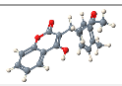
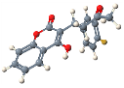
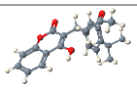
Table 1. Predictions of the pIC_{50} values.

Nº	**3D Structures**	**IUPAC name**	**Predicted pIC_{50}**
1		4-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2-methoxy-2-methylpyrano[3,2-c]chromen-5(2H)-one	3,881001
2		3,4-dihydro-2-methoxy-2-methyl-4-(4-nitrophenyl)pyrano[3,2-c]chromen-5(2H)-one	3,951100
3		3,4-dihydro-2-methoxy-4-(4-methoxyphenyl)-2-methylpyrano[3,2-c]chromen-5(2H)-one	3,829745
4		3,4-dihydro-2-methoxy-2-methyl-4-phenylpyrano[3,2-c]chromen-5(2H)-one	3,943571

5		3,4-dihydro-2-methoxy-2-methyl-4-p-tolylpyrano[3,2-c]chromen-5(2H)-one	3,946035
6		4-(4-fluorophenyl)-3,4-dihydro-2-methoxy-2-methylpyrano[3,2-c]chromen-5(2H)-one	3,959642
7		4-(4-tert-butylphenyl)-3,4-dihydro-2-methoxy-2-methylpyrano[3,2-c]chromen-5(2H)-one	3,902871
8		4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one	3,282264
9		4-hydroxy-3-(1-(4-methoxyphenyl)-3-oxobutyl)-2H-chromen-2-one	3,253075
10		3-(1-(4-(trifluoromethyl)phenyl)-3-oxobutyl)-4-hydroxy-2H-chromen-2-one	3,253213
11		4-hydroxy-3-(1-(4-nitrophenyl)-3-oxobutyl)-2H-chromen-2-one	3,281052
12		4-hydroxy-3-(3-oxo-1-p-tolylbutyl)-2H-chromen-2-one	3,283764
13		3-(1-(4-fluorophenyl)-3-oxobutyl)-4-hydroxy-2H-chromen-2-one	3,293596
14		3-(1-(4-tert-butylphenyl)-3-oxobutyl)-4-hydroxy-2H-chromen-2-one	3,269560
15		4-hydroxy-2H-chromen-2-one	3,365531

3. Materials and Methods

Data: Experimental results of the scavenging ability of the DPPH• radical (expressed as IC_{50}) for 1329 molecules extracted over 170 scientific reports in the literature; thus yielding a comprehensive and diverse database of compounds for the mathematical analysis. All the structures were optimized using CORINA software. The response variable values (IC_{50}) were transformed to their corresponding pIC_{50} values.

Molecular Descriptors: The parameterization of the structures was performed using 3224 molecular descriptors implemented in the DRAGON software. A wrapper based variable selection procedure was used to obtain a subset of 14 variables for the ANN building: MATS2e, BELe6, HATS3u, H2v, R7v, nN-N, nImidazoles, C-005, C-020, O-057, O-060, GVWAI-50, B02 [O-S] and B07 [O-S]*.

Development of ANN model: The QSAR model was develop using as chemometric tool a Multilayer Perceptron Neural Network implemented in STATISTICA 8.0 software. For the modeling, a Broyden-Fletcher-Goldfarb-Shanno training algorithm was used as the optimization method. The following network architecture was established: fourteen inputs; eight neurons in hidden layer and one output.

Predictions: The coumarin derivatives were optimized following the same configuration previously used and the corresponding MD computed.

In vitro DPPH• assay: The free radical scavenging activity of the 4-hydroxycoumarin was measured using the stable DPPH• radical, according to Blois´s method [16]. Briefly, 0.1 mM solution of DPPH• in methanol was prepared and this solution (1 mL) was added to a sample solution in methanol (3 mL) at different concentrations (150–750 μg/mL). The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. BHT was use for comparison. The procedure was triplicate to ensure the results. The capability to scavenge the DPPH• radical was expressed as IC_{50} (concentration of antioxidant that produces 50% of absorbance inhibition).

4. Conclusions

The scavenging capacity of the DPPH• radical is one of the most extended method to evaluate the *in vitro* antiradical activity. An MLP neural network was constructed to relate the structure of 1329 molecules and their antiradical activity. The obtained model showed adequate fitness and a good predictive power and was thus used to predict the antioxidant activity of 15 coumarin derivatives. The *in silico* predictions were further corroborated by an *in vitro* assay for one of the molecules considered as the reference for this set of compounds, and the obtained IC_{50} value was similar to the value predicted by the MLP model.

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Author Contributions

E.G.J. and S.J.B. are responsible for the model construction and evaluation. M.E.J.R. is responsible for the *in vitro* assay. The French team, A.M.R. and M.S.-I.V., provide the coumarin derivatives structures from their chemical library. All authors contributed to the drafting and revision of the article and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.

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*To see the description about each variable, please consult the Handbook of Molecular Descriptors

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