QUANTITATIVE STRUCTURE-ANTIOXIDANT ACTIVITY RELATIONSHIP OF QUERCETIN AND ITS NEW SYNTHETISED DERIVATIVES

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Abstract: Interest in the biological activity of the flavonoids increases due to the potential health benefits of these polyphenolic components of foodstuff. Our research investigates biological properties of the flavonoids and their new synthetized derivatives, focuses on the relationship between their antioxidant activity and their chemical structures.
Quantitative structure-activity relationship (QSAR) attempts to correlate chemical structure with biological activity using statistical approaches. It is the process by which chemical structure of a molecule is quantitatively correlated with a well defined process, such as biological activity, that can be expressed quantitatively as the concentration of a substance required to give a certain biological response. When physicochemical properties or structures are expressed by numbers, the mathematical relation can be formed between the two. The mathematical expression can then be used to predict the biological response of other chemical compounds.
QSARs represent predictive models derived from application of statistical tools correlating antioxidant activity (including desirable therapeutic effect and undesirable side effects) of chemicals with descriptors representative of molecular structure and properties. Obtaining a good QSAR model depends on many factors, such as the quality of biological data, the choice of descriptors and statistical methods. Any QSAR modeling should ultimately lead to statistically robust models capable of making accurate and reliable predictions of biological activities of new untested compounds.

Key words: QSAR, flavonoids, antioxidant activity

1. Introduction

Flavonoids are large group of polyphenolic compounds that are widely distributed in nature. Over 6000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages and are dietary antioxidants. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health (Pick *et al*., 2011; Tian *et al*., 2009). Polyphenols easily protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress induced cytotoxicity (Duthie *et al*., 1997; Skaper *et al*., 1997). In addition, these compounds show a wide spectrum of action involving antitumor, antiviral, antibacterial, cardioprotective, prooxidant and antimutagenic activity (Caballero, 2010; Fan *et al*., 2011; Markovits *et al*., 1989).

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According to the chemical structure, flavonoids can be categorized into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones, etc. The basic structure of flavonoids allows a variety of substitution patterns in the benzene rings. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most common dietary flavonol, is a potent antioxidant, because it has all the right structural features for free radical scavenging activity.

Our work concerns about new synthetised derivatives of quercetin, that may be potentially useful in the prevention of human disease attributed to free radical damage. The observation of antioxidant activity referring to various substituents may lead to the discovery or synthesis of novel flavonoids as preventive or therapeutic agents against human diseases associated with free radicals. A computing technique of artificial neural networks (ANN) was applied for prediction of antioxidant activity of quercetin derivatives.

2. Materials and methods

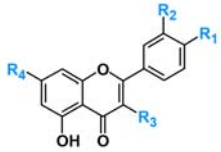
The structures of all 10 investigated derivatives of quercetin are listed in Table 1. The main characteristic of an antioxidant is its ability to trap free radicals. DPPH (Dawidowitz *et al.*, 2012) and ABTS (Floegel *et al.*, 2011) test were applied for determination of the inhibitory potencies of flavonoids.

A rapid, simple and inexpensive method to measure antioxidant activity of compounds involves the use of the free radical, 2,2-diphenyl-1-picrylhydrazyl, DPPH (Sigma-Aldrich GmbH, DE). (S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox (Sigma-Aldrich GmbH, Germany, DE), was used as a reference standard for this purpose. 12 mg of DPPH was diluted in 100 ml of 100% methanol (Vitrum spol s r.o., Czech Republic, CZ). Trolox and 10 new synthetised derivatives as well as quercetin were diluted in 100% dimethylsulfoxide, DMSO (Sigma-Aldrich GmbH, Germany, DE), to appropriate concentration (312.5 μM - 5 mM). 50 μl of sample, 100 μl of 100% methanol and 100 μl of DPPH solution were pipetted into microplate well (Greiner Bio-One GmbH, Germany, DE). The reaction of samples with DPPH solution lasted for 10 min at room temperature in dark. After that, the absorbance changes were measured spectrophotometrically (Multiscan FC Microplate Photometer, Thermo Fisher Scientific Inc., United States, US) at 520 nm. Inhibitory concentrations (IC_{50}) were expressed as the quantity of sample necessary to react with one half of the DPPH (Table 2).

ABTS test was also used to determine the total antioxidant activity of synthetic compounds. The assay measures ABTS radical cation formation induced by metmyoglobin and hydrogen peroxide. Trolox serves as a positive control inhibiting the formation of the radical cation in a dose dependent manner.

Quercetin, its derivatives and trolox were diluted in dimethylsulfoxide (DMSO) to appropriate concentrations (from 8 mM to 15.6 μM). Cationradical $ABTS^{\cdot+}$ was prepared by reaction between 7 mM 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) in phosphate buffered saline (0,1 M, pH 7.4) and 2.45 mM potassium

Table 1. Structures of quercetin and investigated quercetin derivatives.



	R_1	R_2	R_3	R_4
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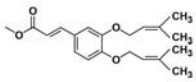
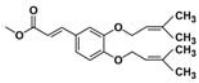
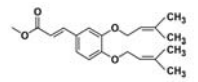
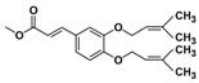
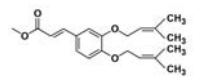
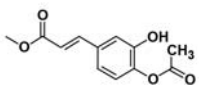
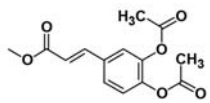
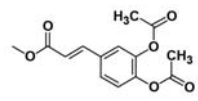
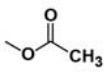
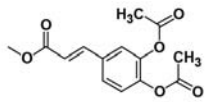
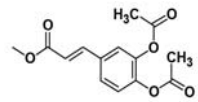
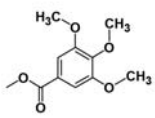
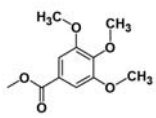
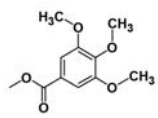
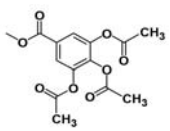
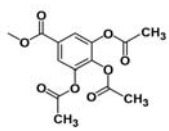
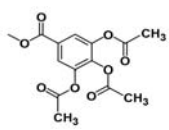
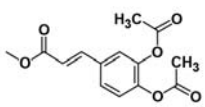
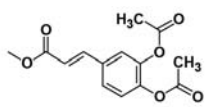
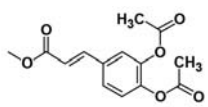
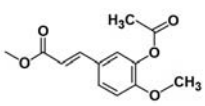
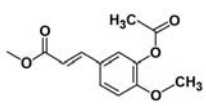
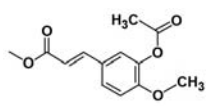
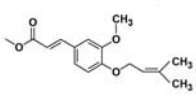
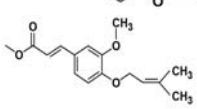
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
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persulfate in phosphate buffered saline (0.1 M, pH 7.4) in the rate of 1:1. This mixture stayed at room temperature in the dark for 12 hours. Solution of cationradical $ABTS^{\cdot+}$was diluted with DMSO (1.5 ml of $ABTS^{\cdot+}$ was pipetted into 60 ml of DMSO) to get an absorbance of 0.700 at 734 nm. Then 1.95 ml of diluted $ABTS^{\cdot+}$ was added to 0.05 ml of sample. Reaction mixture was incubated 7 min at room temperature in the dark. Thereafter the absorbance was measured at 734 nm. The inhibition percentage was calculated for each concentration relative to a blank absorbance (DMSO). Value of IC_{50} was stated (the concentration, which eliminates the half of radical present) for each sample (Table 2).

Table 2. Antioxidant activity determined by the radical scavenging activity of antioxidants against the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

No.	Name	IC_{50} ± SD (μM)	
		ABTS	DPPH
1.	quercetin	2.5 ± 0.1	16.2 ± 1.1
2.	tri(diprenylcaffeoyl) quercetin	8.4 ± 0.6	> 200.0
3.	di(diprenylcaffeoyl) quercetin	5.5 ± 0.1	160.9 ± 13.2
4.	di(diacethylcaffeoyl)-mono(monoacethylcaffeoyl) quercetin	10.4 ± 0.7	> 200.0
5.	monoacethyl-di(diacethylcaffeoyl) quercetin	4.1 ± 0.4	29.2 ± 0.2
6.	tri(trimethylgalloyl) quercetin	16.0 ± 2.1	> 200.0
7.	tri(triacethylgalloyl) quercetin	32.4 ± 2.8	52.8 ± 4.5
8.	tri(acethylcaffeoyl) quercetin	5.8 ± 0.6	35.4 ± 2.7
9.	tri(acethylferuloyl) quercetin	6.1 ± 0.4	> 200.0
10.	di(prenylferuloyl) quercetin	5.6 ± 0.6	82.8 ± 12.8
11.	monoacethylferuloyl quercetin	24.9 ± 1.5	> 200.0
12.	trolox	8.7 ± 0.5	34.7 ± 1.0

For QSAR purposes (Kartasasmita *et al*., 2009; Nemeček *et al*., 2006; Todeschini, 2009), the 3D structures of quercetin and its derivatives were first constructed using software Hyperchem v. 8.0.6 (Hypercube Inc., United States, US), then were optimized using Austin Model 1 (AM1). For optimization of each structure 2000 maximum iterations were applied, followed by conjugate gradient minimization to a Root Mean Square (RMS) energy gradient of 0.1 kcal/(Å mol). After optimization, seven QSAR properties were obtained for each structure (Table 3). The inhibitory concentrations (IC_{50}) were predicted using the artificial neural network techniques (Statistica v. 8; StatSoft Inc., United States, US) and these were compared with measured values (Table 4). ANN is modeled after the (hypothesized) processes of learning in the cognitive system and the neurological functions of the brain, and capable of predicting new observations (on specific variables) from other observations (on the same or other variables) after executing a process of so-called learning from existing data (Bocaz-Beneventi *et al*., 2002; Gasteiger, 2006; Kvasnička *et al*., 1997). The artificial neural network itself consists of neurons arranged in array-like structures known as the input, hidden and output layers. Each layer consists of at least one

neuron. Each neuron is represented by a mathematical function which transforms the incoming signals from the layer below into an outgoing signal. This process is repeated till the artificial neural network output (prediction) is produced (Kruzlicova *et al*., 2009).

3. Results and discussion

Ten derivatives of quercetin were characterized by seven descriptors, which have been used in the ANN training process. A large amount of networks with different architectures were examined during the training step. A variety of algorithms for different neural network types were automatically tested and the best alternatives were selected. The number of input neurons was set by the number of descriptors (7); output neuron (1) represented calculated IC_{50} value (expressed in $\log(1/IC_{50})$ form (Thakur *et al.*, 2004)). The optimal choice of hidden neurons depends on the problem domain and can be related to the number of the inputs. The final number was found by examining several types of the three layer perceptron (3-MLP) with regard to the corresponding final root mean squared (RMS) error.

Table 3. Values of QSAR properties for quercetin (no. 1) and ten derivatives (no. 2-11) calculated after structure optimization by software Hyperchem.

No.	Hydration energy (kcal/mol)	Log P	Refractivity (A3)	Polarizability (A3)	Molecular mass (amu)	LUMO (eV)	HOMO (eV)
1.	-32.82	0.28	73.43	28.54	302.24	-1.069324	-8.770832
2.	-18.77	12.55	348.48	131.44	1197.39	-1.004567	-8.677681
3.	-23.21	8.46	257.46	97.14	899.00	-0.997896	-8.591627
4.	-30.79	3.80	252.35	96.32	998.86	-1.310604	-8.890491
5.	-23.64	2.27	209.11	79.99	836.72	-1.299082	-8.889960
6.	-30.57	3.09	222.43	85.53	884.80	-0.969415	-9.153621
7.	-29.54	0.86	264.45	102.82	1136.89	-1.523262	-9.381587
8.	-29.20	3.59	261.79	100.08	1040.90	-1.572512	-9.412628
9.	-28.45	4.33	247.78	94.31	956.87	-1.367721	-9.197675
10.	-25.59	5.97	219.23	82.84	790.82	-0.967023	-8.808840
11.	-30.67	1.63	132.88	50.47	520.45	-1.185066	-8.984444

Input neurons received the input data variables for each QSAR property, the output neuron provided predicted value of the studied objects (inhibitory concentration). Statistica's Automatic Network Search function (ANS) was used for primordial ANN building. Data set was split into 60:40 (60% of samples were utilizing the model and 40% of samples were used for further validation of the model). Multi-layer perceptron (MLP) network type was chosen with the specification from 1 to 3 hidden neurons. The learning process was initialised by the BFGS (or Quasi-Newton) training algorithm. The number of cycles (epochs) was 200 (the network error was calculated

in each training cycle and was used to adjust the weights so that the error was further reduced). Weight decay value was set to 10^{-6} for hidden layer and 10^{-5} for output layer. A number of 1000 neural networks were trained for (a) prediction of IC_{50} obtained from ABTS test as well as for (b) prediction of IC_{50} determined by DPPH test. The network with best performance was found for each case.

The optimal network architecture MLP 7-3-1 was found according to the root mean squared error in case of $\log(1/IC_{50})_{ABTS}$ calculation. The hyperbolic tangent function (tanh) was used for hidden neurons activation as well as for output neuron. Training performance reached 0.9996 with the error 0.0000 and the test performance was 1.0000 with the validation error 0.0124.

In case of neural network for $\log(1/IC_{50})_{DPPH}$ prediction, MLP 7-3-1 with exponential function for hidden neurons activation and with logistic function for output neuron activation was the network with the best prediction performance. Training performance was 0.9969 with training error 0.0005; validation performance was 0.8591 with the validation error 0.0268. Computed values for recalculated IC_{50} are listed in Table 4.

Table 4. Comparison of measured and predicted values of antioxidant activity expressed as inhibitory concentration (IC_{50}). ABTS and DPPH tests were used for experimental determination of IC_{50} values. And calculations of predicted values were made by using artificial neural networks.

Name	IC_{50} (μM)					
	ABTS test			DPPH test		
	measured	predicted	RSD (%)	measured	predicted	RSD (%)
tri(diprenylcaffeoyl) quercetin	8.4	8.3 [b)]	-1.0	200.0	200.0 [b)]	0.0
di(diprenylcaffeoyl) quercetin	5.5	5.3 [a)]	-3.4	160.9	153.4 [a)]	-4.7
di(diacethylcaffeoyl)-mono(monoacethylcaffeoyl) quercetin	10.4	6.7 [b)]	-35.4	200.0	200.0 [b)]	0.0
monoacethyl-di(diacethylcaffeoyl) quercetin	4.1	4.1 [a)]	0.0	29.2	29.7 [a)]	1.7
tri(trimethylgalloyl) quercetin	16.0	16.1 [a)]	0.7	200.0	200.0 [a)]	0.0
tri(triacethylgalloyl) quercetin	32.4	32.3 [a)]	-0.2	52.8	52.5 [a)]	-0.6
tri(acethylcaffeoyl) quercetin	5.8	6.7 [b)]	15.8	35.4	72.5 [b)]	104.8
tri(acethylferuloyl) quercetin	6.1	6.2 [b)]	2.0	200.0	117.8 [b)]	-41.1
di(prenylferuloyl) quercetin	5.6	5.8 [a)]	3.6	82.8	95.1 [a)]	14.8
monoacethylferuloyl quercetin	24.9	24.7 [a)]	-0.9	200.0	198.7 [a)]	-0.7

[a)] samples were used for building of the prediction model
[b)] samples were used for validation of the model

The fact, that models are suitable for prediction of antioxidant activity of untested derivatives, is confirmed by predictive squared correlation coefficient Q^2 (Consonni *et al.*, 2010). The value of Q^2 was 0.9745 for $\log(1/IC_{50})_{ABTS}$ predictions and 0.9907 for $\log(1/IC_{50})_{DPPH}$ predictions.

It is important to notice, that the use of ten derivatives is not a lot for data set build up, thus better results can be obtained with the study of data matrix containing more derivatives prepared in the future.

4. Conclusions

It has been demonstrated that technique of artificial neural network can be a very useful tool for study of relationship between structure and antioxidant activity. They provide a new alternative to the most commonly used methods for developing predictive models, which are often limited by strict assumptions of normality, linearity, variable independence etc. Logarithm of inhibitory concentration is one of the most common forms of the antioxidant activity used as dependent variable for the neural network model. Main advantages of these modern approaches are (a) robust performance in dealing with noisy or incomplete input patterns, (b) ability to detect complex nonlinear relationships between dependent and independent variables, (c) ability to detect all possible interactions between predictor variables, (d) and the availability of multiple training algorithms. These advantages make ANNs superior and applicable in various predictions for the most of biological and clinical data.

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